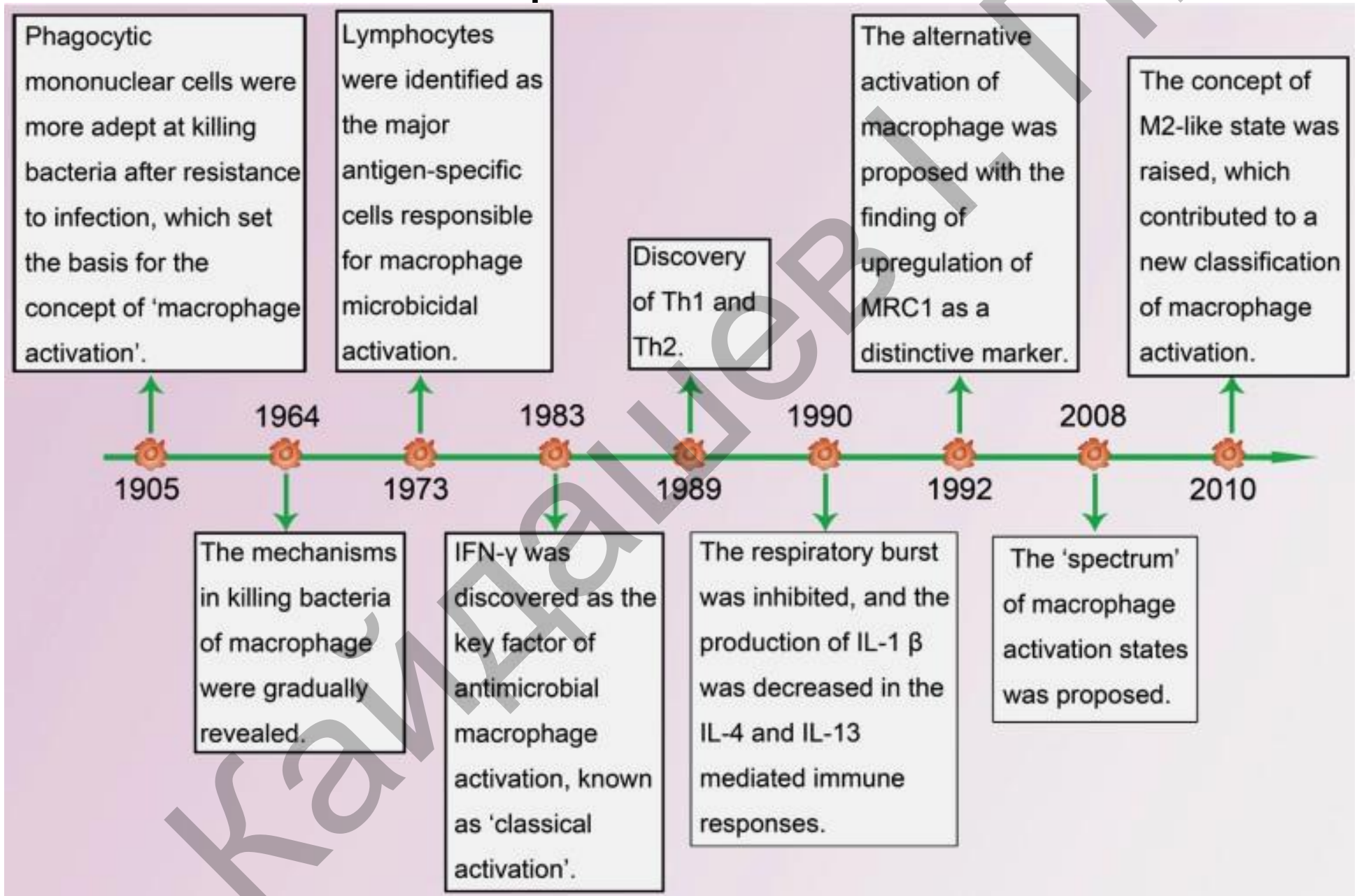


# Macrophage subpopulations and immunoregulation

**Prof. Igor P. Kaidashev**  
*Ukrainian Medical  
Stomatological Academy,  
Poltava, Ukraine*

# Timeline: advance in research of macrophage polarization



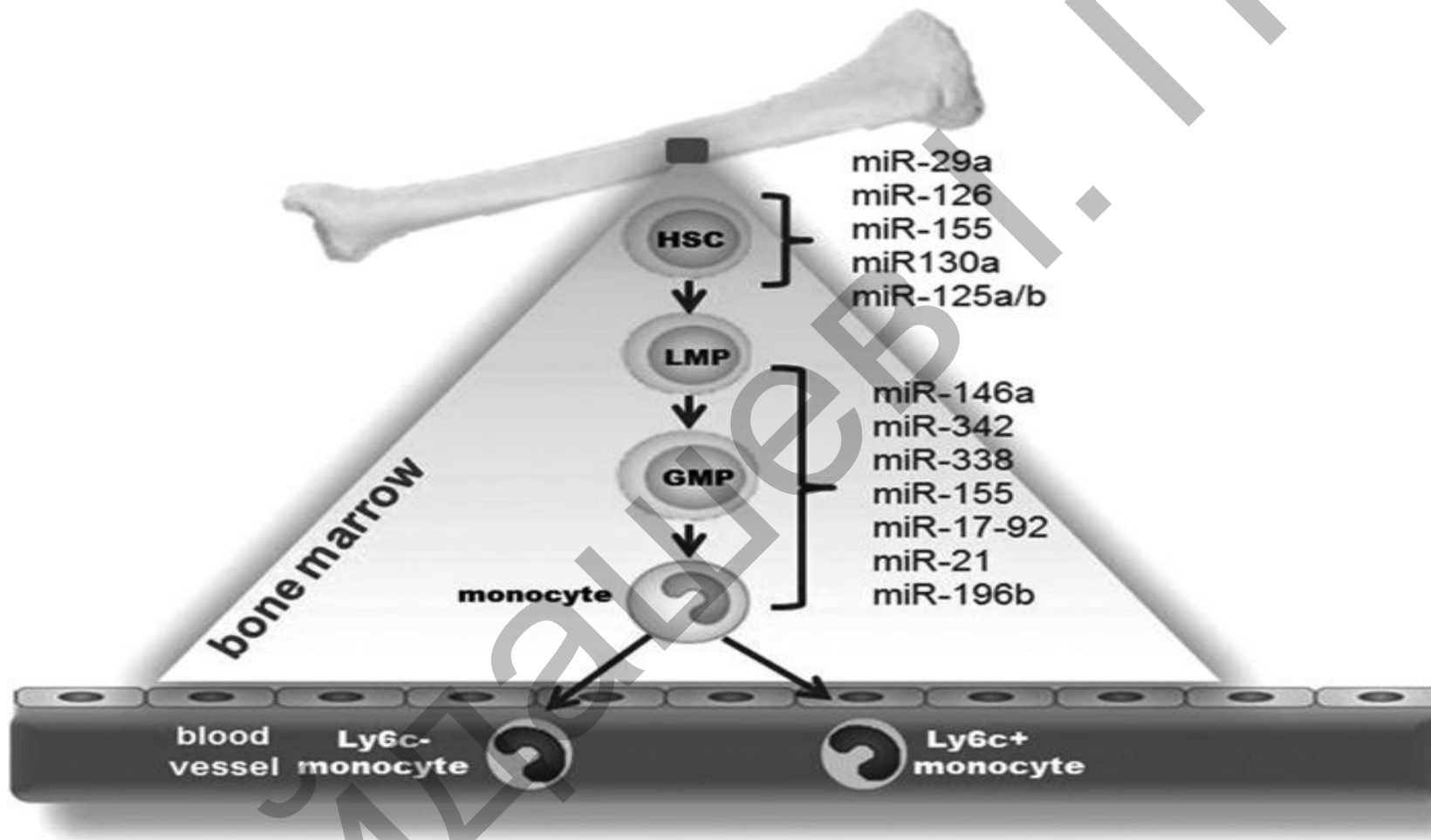
# Mononuclear Phagocytic System (MPS)

- Lineage-committed bone marrow precursors
- Circulating monocytes (Mo)
- Resident macrophages ( $M\phi$ )
- Dendritic cells

# Monocytes origin

- Hematopoietic stem cells (HSCs);
- Common myeloid progenitor (CMP);
- Granulocyte-macrophage progenitor (GMP);
- Common macrophage and DC precursor (MDP);
- Committed monocyte progenitor (cMoP)
- Monocytes.

# Involvement of microRNAs (miRNAs) in monocyte/macrophage development



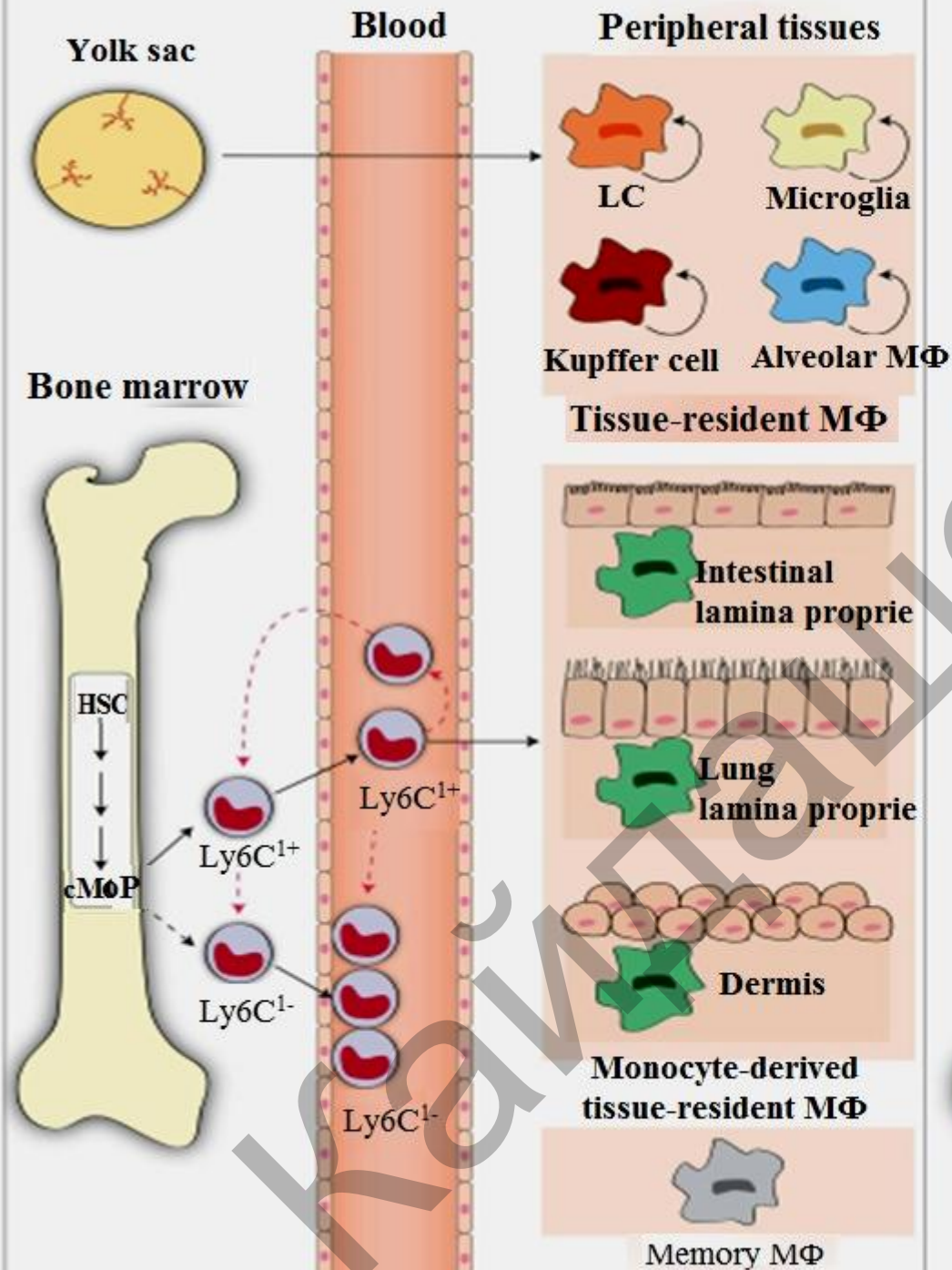
Circulating monocytes are primarily considered the precursors of macrophages. Monocytes originate from adult hematopoietic stem cells (HSCs) that under favorable conditions differentiate to → lymphoid–myeloid progenitor (LMP) → granulocyte–monocyte progenitor (GMP) and via multiple steps mature to monocytes. In blood, two populations of monocytes namely Ly6c+ or Ly6c- have been identified in mice. The monocytes then enter to tissues and differentiate to macrophages.

# Origins of M $\phi$ in fetal and adult tissues

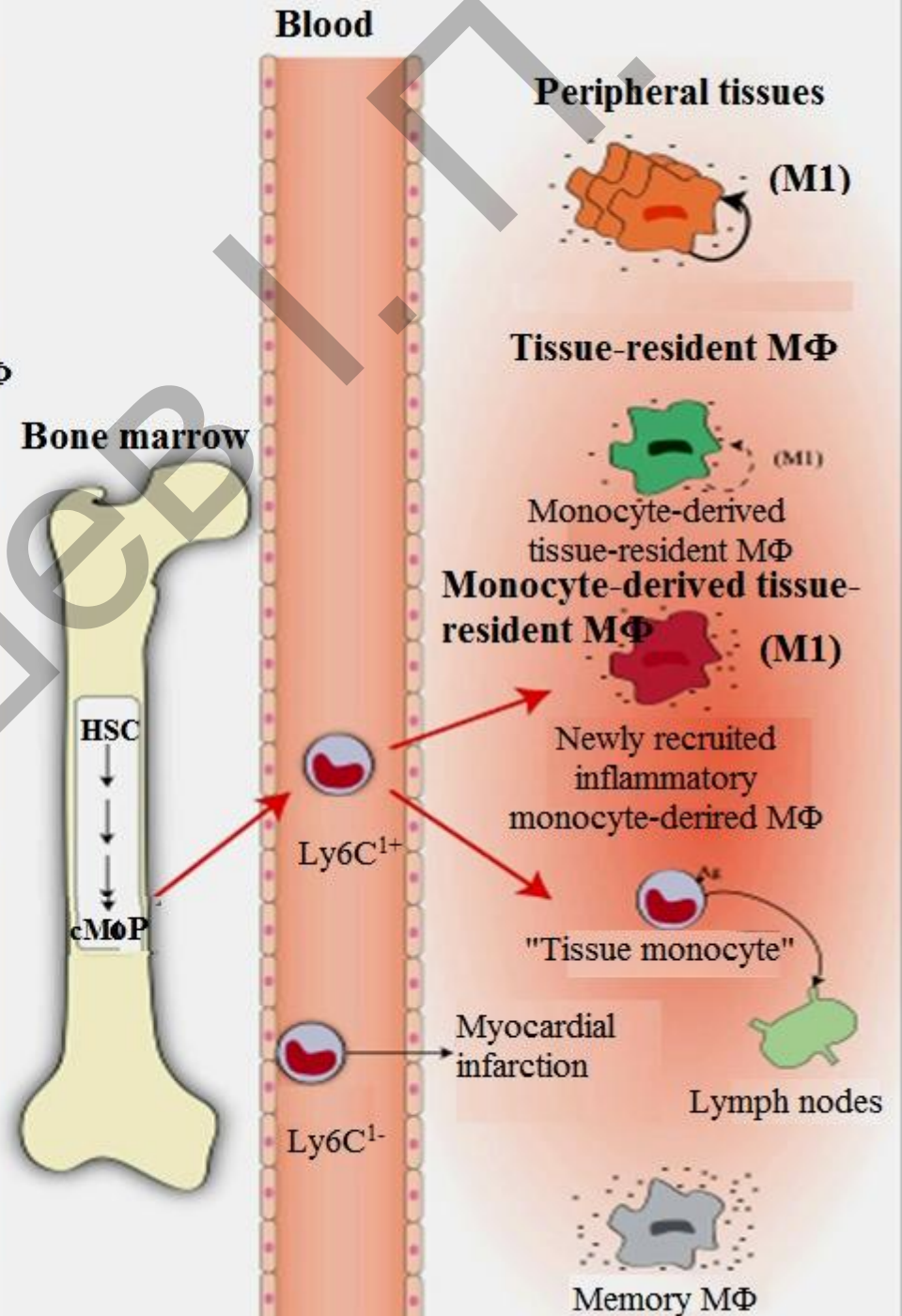
- Yolk sac (giving rise to some tissue-resident yolk sac-derived M $\phi$ ).
- Fetal liver (giving rise to fetal liver-derived M $\phi$ ).
- Bone marrow (giving rise to tissue-resident bone marrow-derived macrophages).



## HOMEOSTASIS



## INFLAMMATION



# Monocytes and MPS

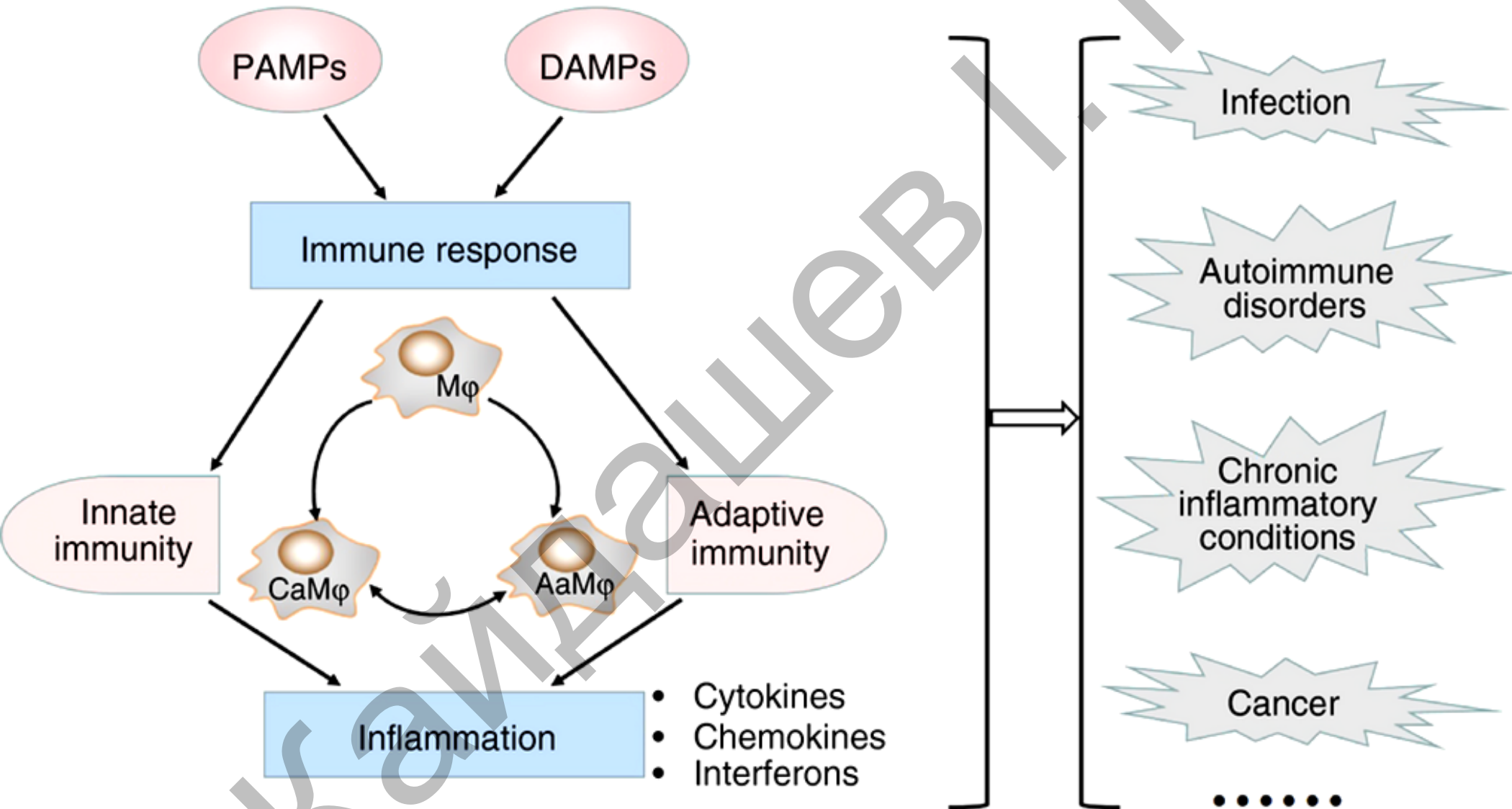
- Systemic reservoir of myeloid precursors for renewal of tissue M $\phi$  and Dcs.
- Circulating monocytes contribute to the repopulation of tissue-resident macrophages under homeostatic conditions in tissues like the lamina propria and healthy skin.
- Some monocytes can enter non-lymphoid organs without obligatory differentiation into M $\phi$  or DCs and are able to present antigens to T cells (in mice).
- Some Mo display a «patrolling» phenotype (via TLR7).



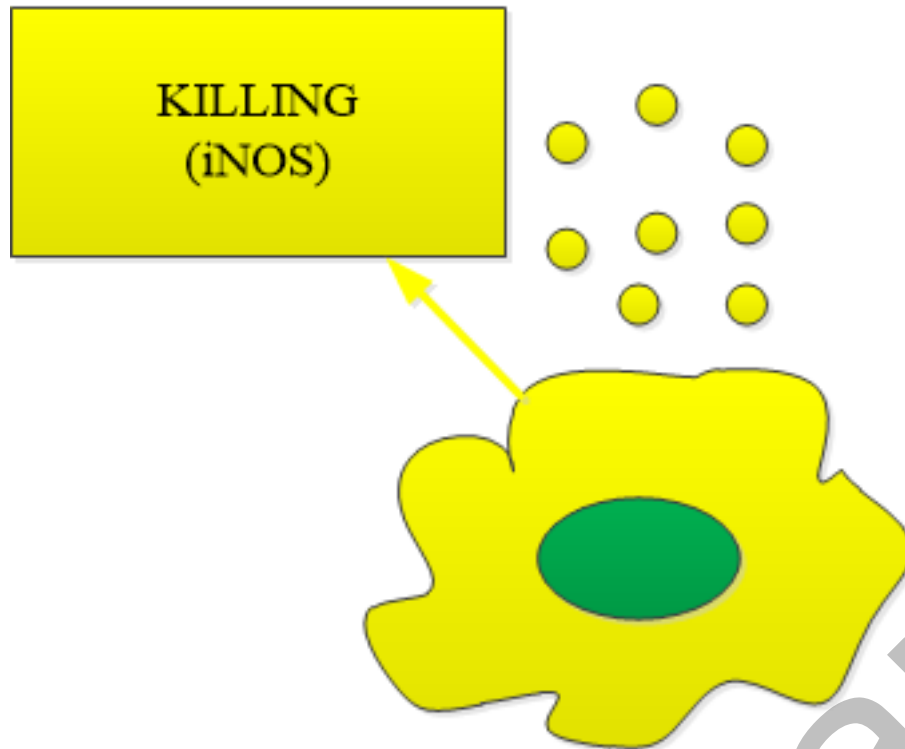
# General functions of macrophages in innate and adaptive immunity

Monocyte/macrophage	
Innate immunity	Opsonic recognition
	Production of pro-inflammatory and anti-inflammatory cytokines
	Release GCSF and GM-CSF
	Excessive release of toxic species (NO, superoxide and MMP)
	Antigen processing, and presentation
Adaptive immunity	Secretion of hydrolytic enzymes
	Cleavage of C3

# Role of macrophage polarization (MP) in immune response and inflammation



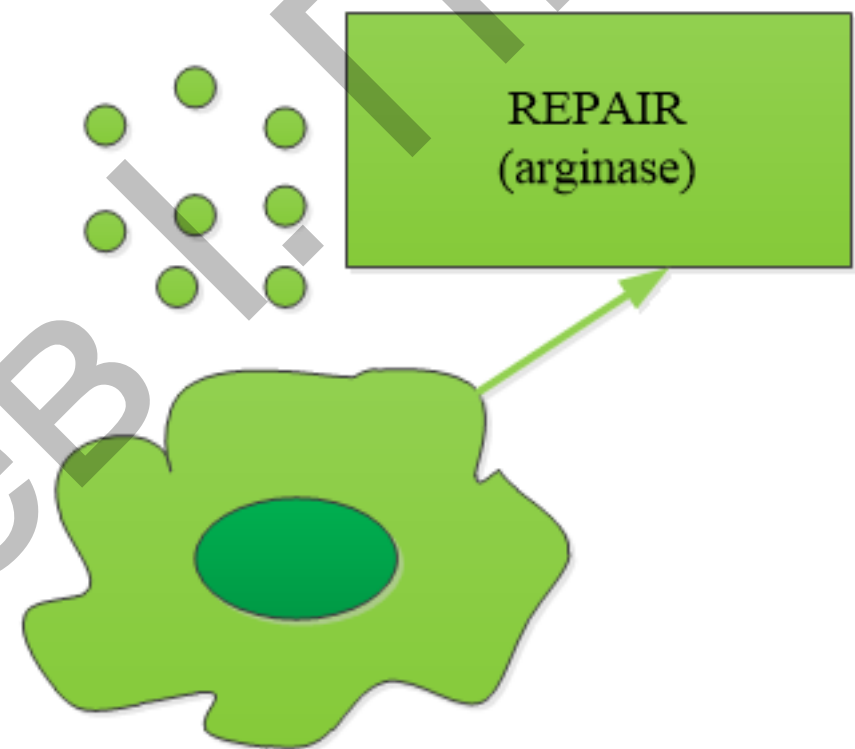
## M1 agonists



## M1 BIOMARKERS

IL-12, TNF, IL6, IL1, IL23  
CXCL8, 9, 10, 11, 16  
CCL2, 3, 5  
CD80, CD86, NOS, ROS,  
MHCII, TLR2/4

## M2 agonists



## M2 BIOMARKERS

IL10, IL2RA  
CXCR1, CXCR2  
CCL17, 18, 22, 24  
CD23, CD163, MR, SR,  
arginase, MHCII

# Multipolar view of M1 and M2a polarization.

M1

M2a

## *polarization factors:*

IFN- $\gamma$ , TLR ligands, hypoxia

IL-4/IL-13, PPAR ligands,  
iron accumulation

## *direct microbicidal mechanisms:*

respiratory burst, NO, ...

Arg1,  
scavenger receptors

## *metabolic profil:*

### *nutritional immunity:*

hypoxia  
iron sequestration  
glucose deprivation

### *nutritional support:*

lipid accumulation,  
glucose and iron availability  
polyamines, ...

## *control of inflammation:*

NO, tryptophan catabolism

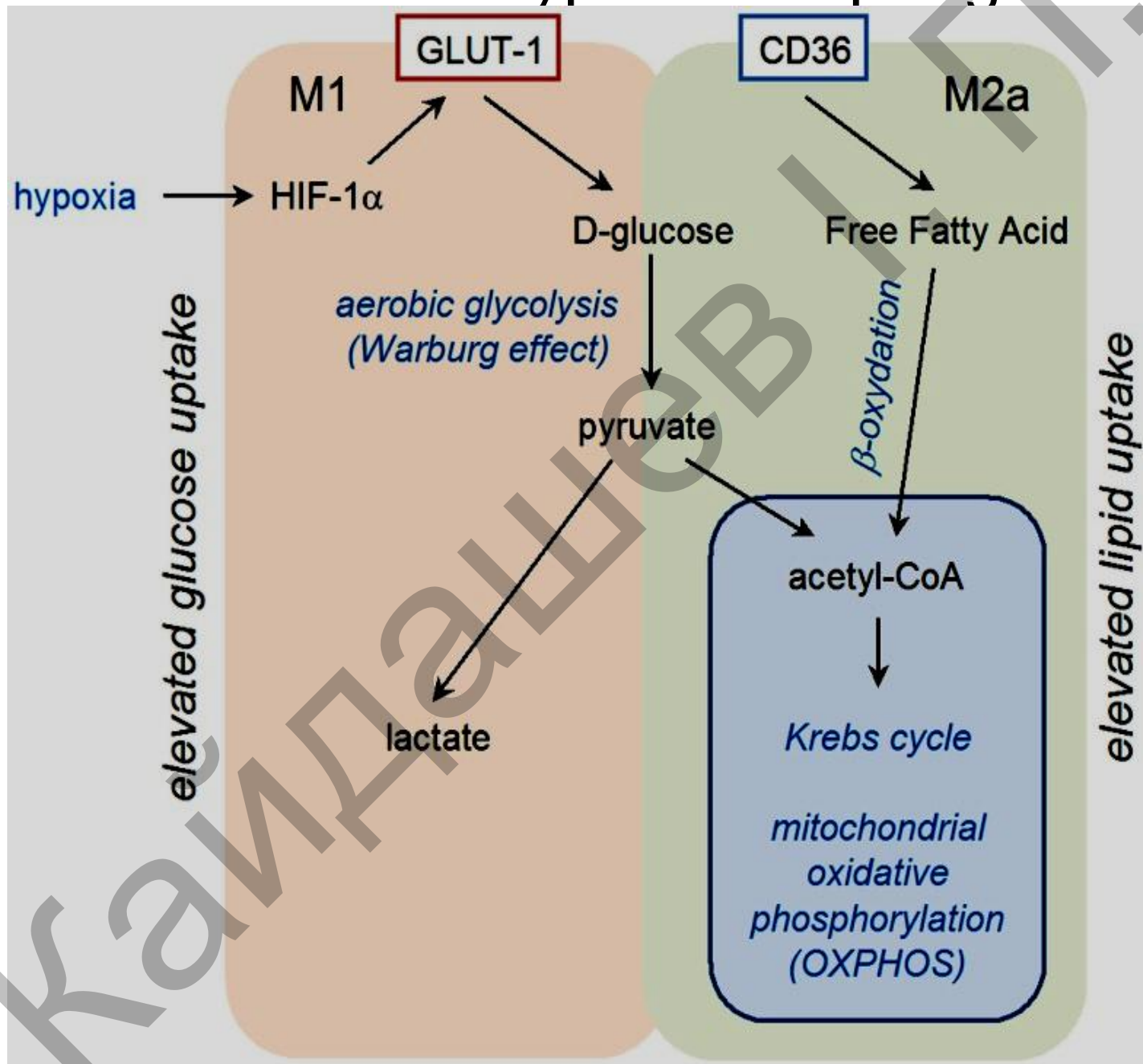
IL-10, CD36, Arg1

Subtypes		
	M1	M2
Inducers	IFN- $\gamma$ , LPS, GM-CSF, oxidative, fatty acid, HMGB1	IL-4, IL-10, IL-13, TGF- $\beta$ , M-CSF, AMP, GC
Transcription factors	NF- $\kappa$ B, STAT1, IRF1, IRF5, HIF-1 $\alpha$ , KLF6	STAT3, STAT6, IRF4, KLF4, PPAR $\gamma$ , cMaf, cMyc
Cytokines	NO, TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IL-12, IL-23	IL-10, TGF- $\beta$
Chemokines	CXCL9, CXCL10, CXCL11	CCL17, CCL18, CCL22
Metabolic enzymes	iNOS, gp91phox and p22phox, ferritin, CP, DMT-1, Nramp-1	Arg-1, Arg-2, ODC, SMO, HO-1, Fpn, TfR
Cell marker	CD80, CD86, TLR2, TLR4, MHC II	CD206, CD163, CD209, CD301, Fizzl, Ym1/2
Functions	inflammatory, microbicidal activity, clearance of pathogen	inflammatory, immune regulators, tissue repair

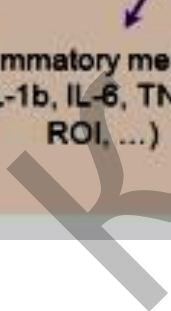
AMP, adenosine monophosphate; Arg, arginase; CCL, chemokine (C-C motif) ligand; CP, ceruloplasmin; CXCL, chemokine (C-X-C) ligand; DMT, divalent metal transporter; Fizz1, resistin-like  $\alpha$ ; Fpn, ferroportin; GC, glucocorticoids; GM-CSF, granulocyte macrophage colony-stimulating factor; HIF, hypoxia inducible factor; HMGB1, high-mobility group box 1; HO-1, hemoxygenase-1; iNOS, inducible nitric oxide synthase; IFN- $\gamma$ , interferon-gamma; IL, interleukin; IRF, interferon regulatory factor; KLF, Kruppel-like factor; LPS, lipopolysaccharides; MHC, major histocompatibility complex; M-CSF, macrophage colony-stimulating factor; NF- $\kappa$ B, nuclear factor  $\kappa$ B; NO, nitric oxide; Nramp, natural resistance-associated macrophage protein; ODC, ornithine decarboxylase; PPAR, peroxisome proliferator-activated receptors; SMO, spermidine oxidase; STAT, signal transducer and activator of transcription; TNF- $\alpha$ , tumor necrosis factor alpha; TGF- $\beta$ , transforming growth factor beta; TfR, transferrin receptor; Ym1, chitinase 3-like 3.



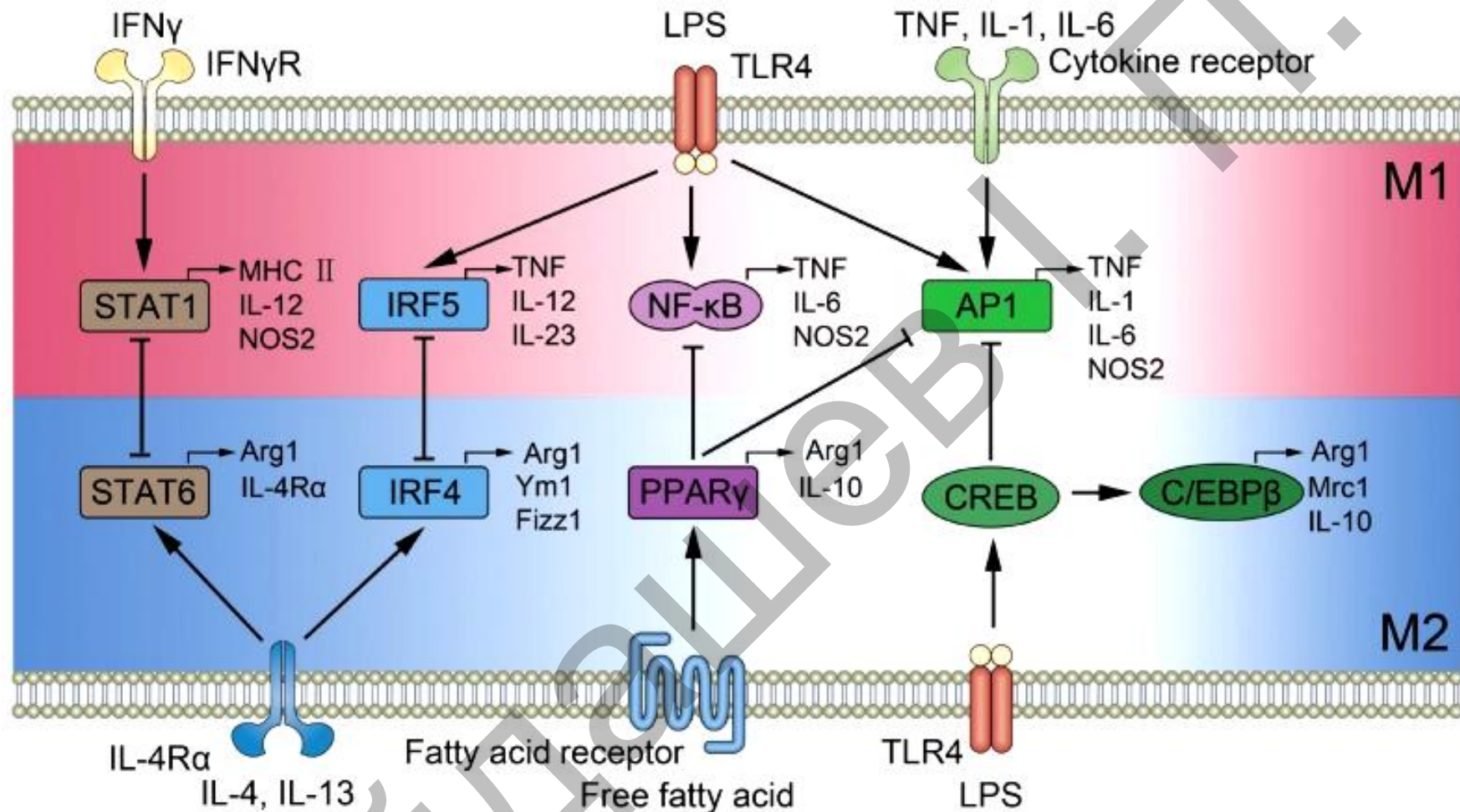
# Schematic representation of polarized metabolism in M1 and M2a-type macrophages



M2a

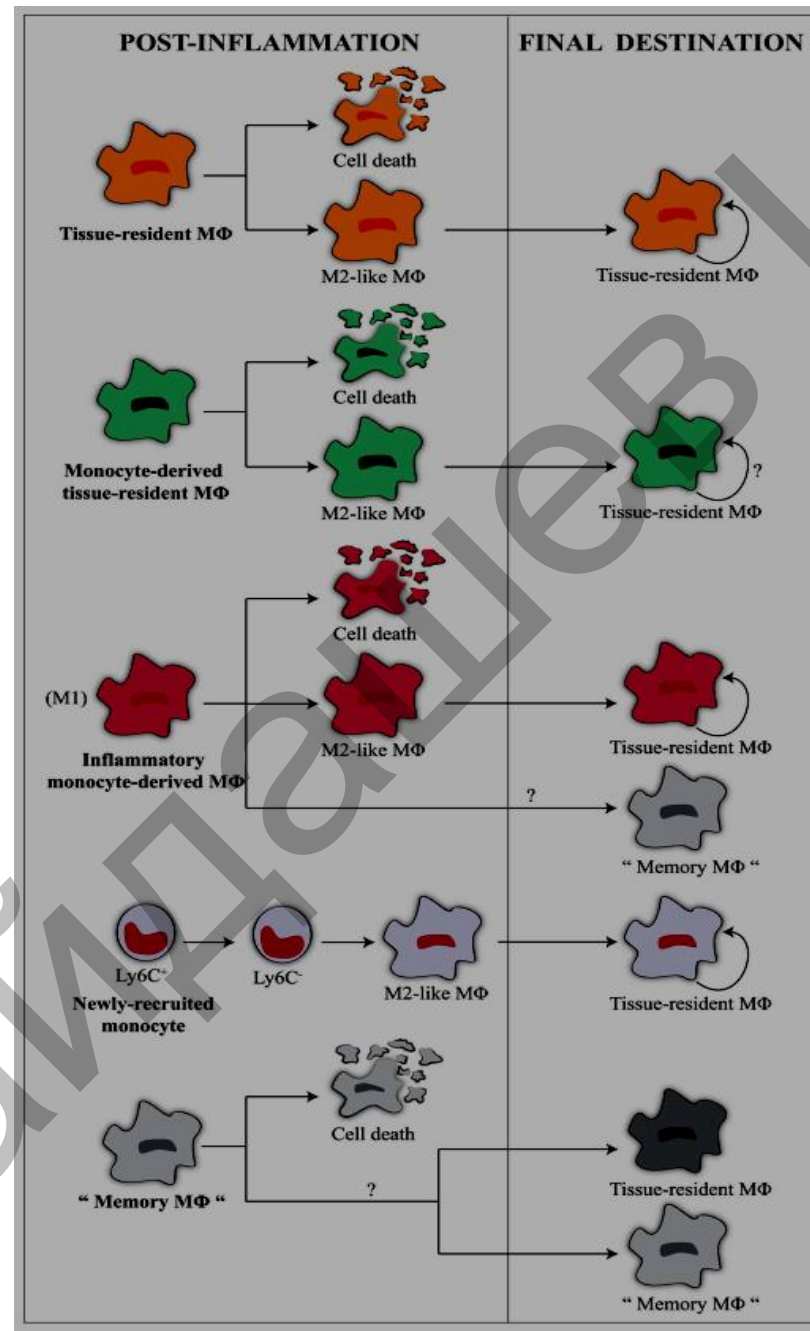


# Signal pathways of macrophage polarization



The figure illustrates several mechanisms underlying macrophage polarization and shows the feedback regulation between M1 and M2 signal pathways. Those include the activation of STAT1 mediated by IFN- $\gamma$  receptor, increase in IRF5, NF- $\kappa$ B, as well as AP1 expression mediated by Toll-like receptor 4 (TLR4), enhanced AP1 expression mediated by cytokine receptor, activation of STAT6 and increased IRF4 mediated by IL-4 receptor, increased level of PPAR $\gamma$  mediated by fatty acid receptor, and enhanced expression in CREB by TLR4. The feedback regulation between M1 and M2 are implemented by STAT1-STAT6, IRF5-IRF4, NF- $\kappa$ B-PPAR $\gamma$ , AP1-CREB, and AP1-PPAR $\gamma$ , and they play essential roles in the initiation, development, and cessation of inflammatory diseases.

# Fate of different Mo/Mf subpopulations after tissue inflammation





# Th1/Th2 Paradigm Extended: Macrophage Polarization as an Unappreciated Pathogen-Driven Escape Mechanism?

[Eric Muraille](#)<sup>1</sup>, [Oberdan Leo](#)<sup>2</sup> and [Muriel Moser](#)<sup>2,\*</sup>

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## Abstract

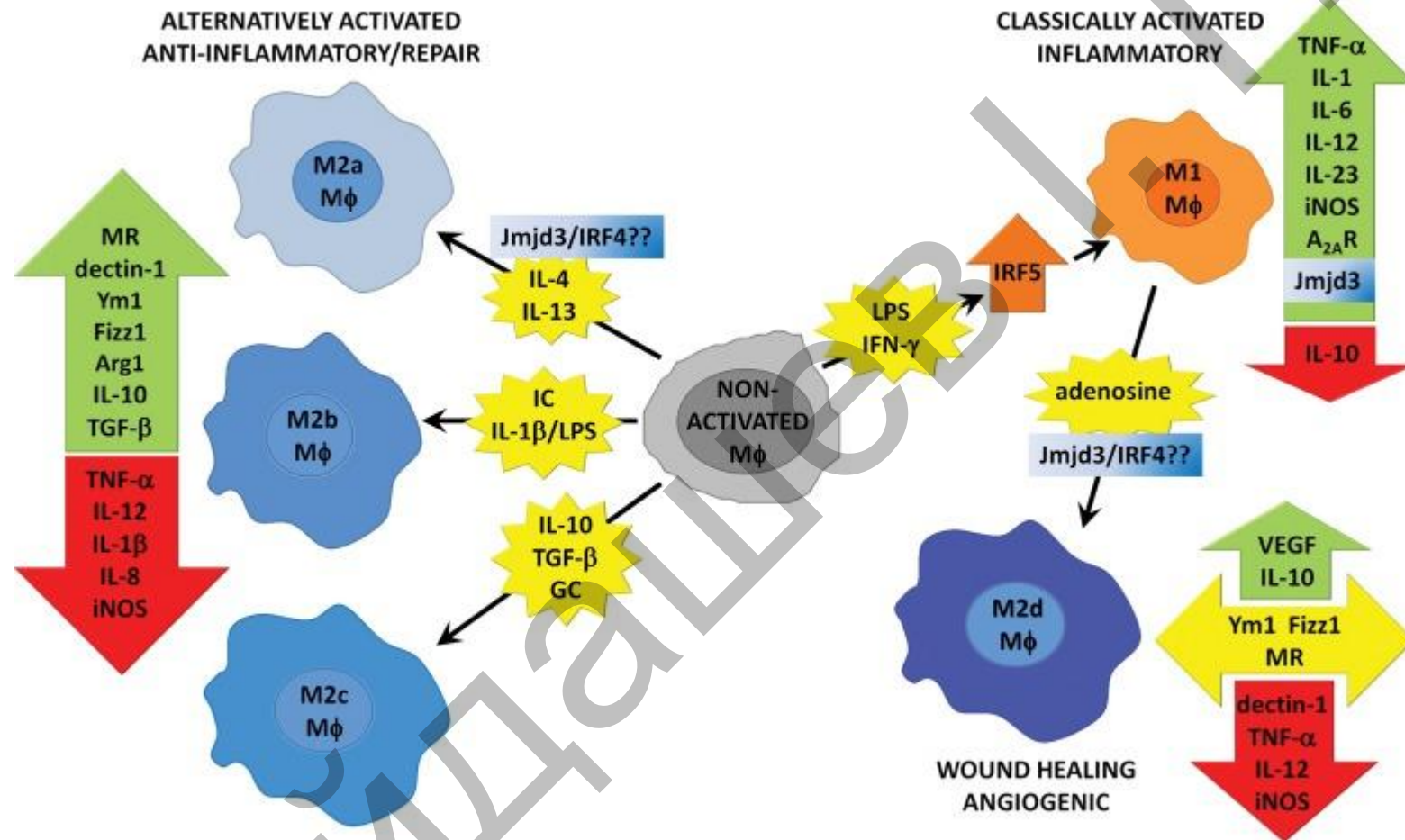
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The classical view of the Th1/Th2 paradigm posits that the pathogen nature, infectious cycle, and persistence represent key parameters controlling the choice of effector mechanisms operating during an immune response. Thus, efficient Th1 responses are triggered by replicating intracellular pathogens, while Th2 responses would control helminth infection and promote tissue repair during the resolution phase of an infectious event. However, this vision does not account for a growing body of data describing how pathogens exploit the polarization of the host immune response to their own benefit. Recently, the study of macrophages has illustrated a novel aspect of this arm race between pathogens and the immune system, and the central role of macrophages in homeostasis, repair and defense of all tissues is now fully appreciated. Like T lymphocytes, macrophages differentiate into distinct effectors including classically (M1) and alternatively (M2) activated macrophages. Interestingly, in addition to represent immune effectors, M1/M2 cells have been shown to represent potential reservoir cells to a wide range of intracellular pathogens. Subversion of macrophage cell metabolism by microbes appears as a recently uncovered immune escape strategy. Upon infection, several microbial agents have been shown to activate host metabolic pathways leading to the production of nutrients necessary to their long-term persistence in host. The purpose of this review is to summarize and discuss the strategies employed by pathogens to manipulate macrophage differentiation, and in particular their basic cell metabolism, to favor their own growth while avoiding immune control.

**Keywords:** macrophage polarization, metabolic switch, amino acid metabolism, hypoxia, iron, PPARs, infection, immune escape strategy

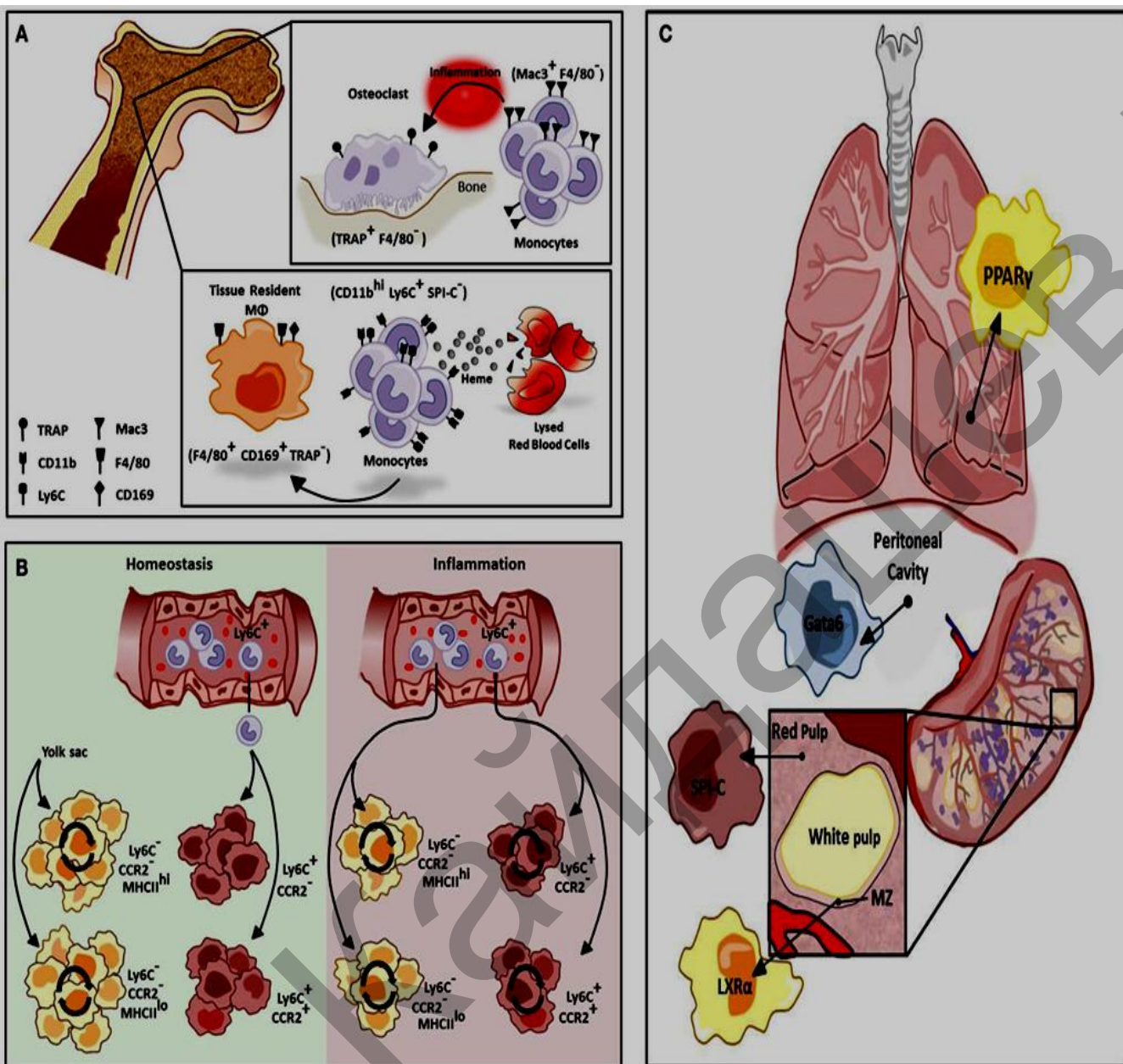


# Pathways of Mφ polarization



Nonactivated Mφs are polarized into distinct phenotypes by specific inducing agents and display typical changes in gene expression. Note that not all inducing agents are included and expression profiles for different M2 Mφ subtypes can differ based on the nature of induction.

# Differentiation of resident macrophages in various physiological states



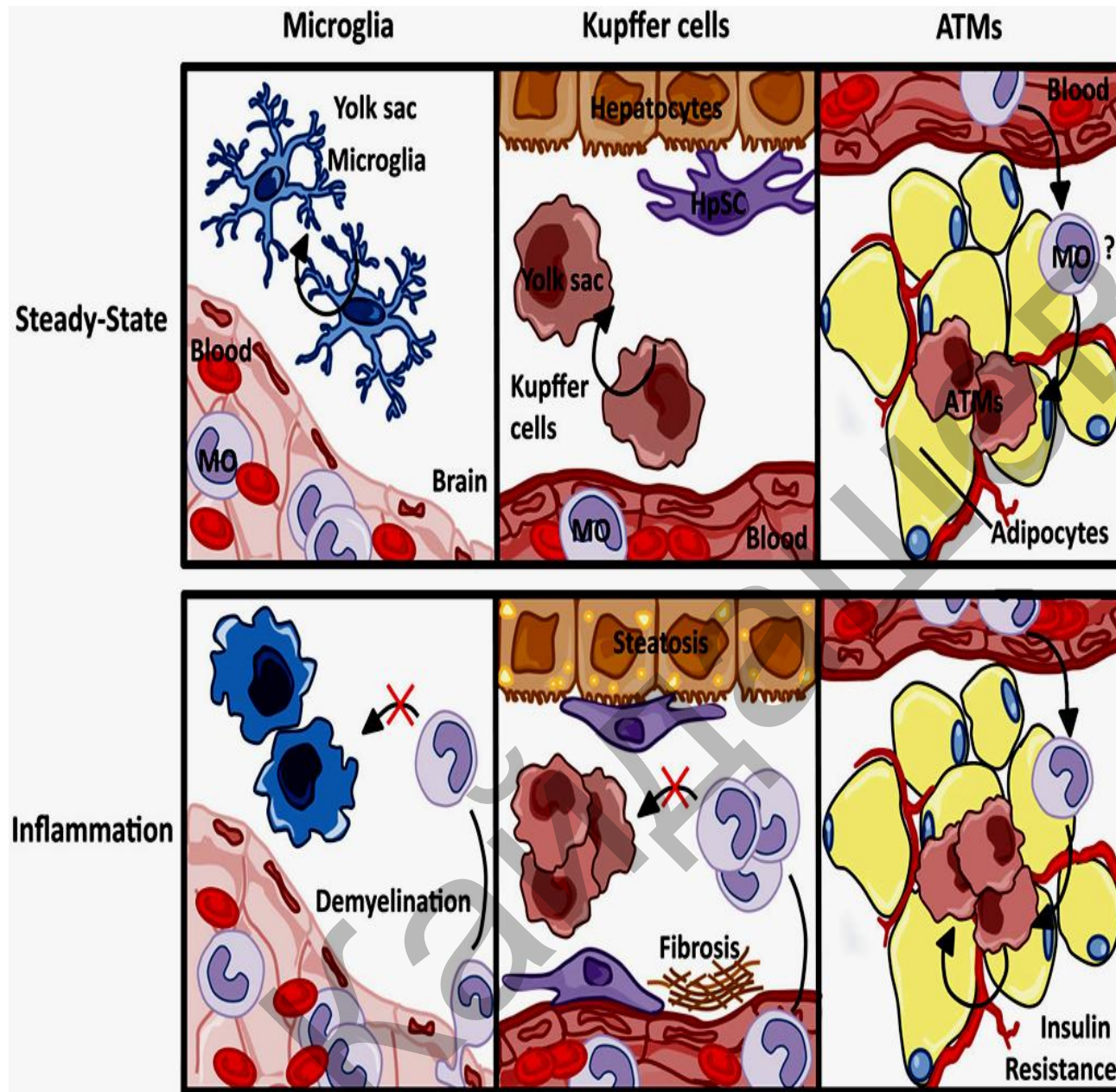
(A) Two tissue macrophage populations reside in bone, F4/80<sup>-</sup> TRAP<sup>+</sup> osteoclasts and F4/80<sup>+</sup> CD169<sup>+</sup> TRAP<sup>-</sup> tissue-resident macrophages (MΦs). Osteoclasts are developmentally dependent on Mac3<sup>+</sup>F4/80<sup>-</sup> monocytes but only during inflammation. Alternatively, tissue-resident macrophages in bone can be maintained by a heme-induced subset of monocytes (CD11b<sup>hi</sup> Ly6C<sup>+</sup> SPI-C<sup>-</sup>).

(B) Cardiac tissue-resident macrophage populations are primarily yolk sac-derived (YS) however a minor subset of the population is derived from fetal liver and HSC-derived progenitors. In homeostasis, primary yolk sac-derived cardiac resident macrophages are maintained through self-renewal but in response to cardiac insult, Ly6Chi monocytes contribute to all four macrophage populations.

(C) Selective transcriptional control plays an important role in the development of different types of macrophage populations. While red-pulp macrophages are dependent on SPI-C activity for development and maintenance, marginal zone (MZ) macrophage differentiation is mediated by LXRα. Alternatively, Gata6 is mandatory for the differentiation and proliferation of peritoneal macrophages while GM-CSF-dependent induction of PPARγ regulate alveolar macrophage development.

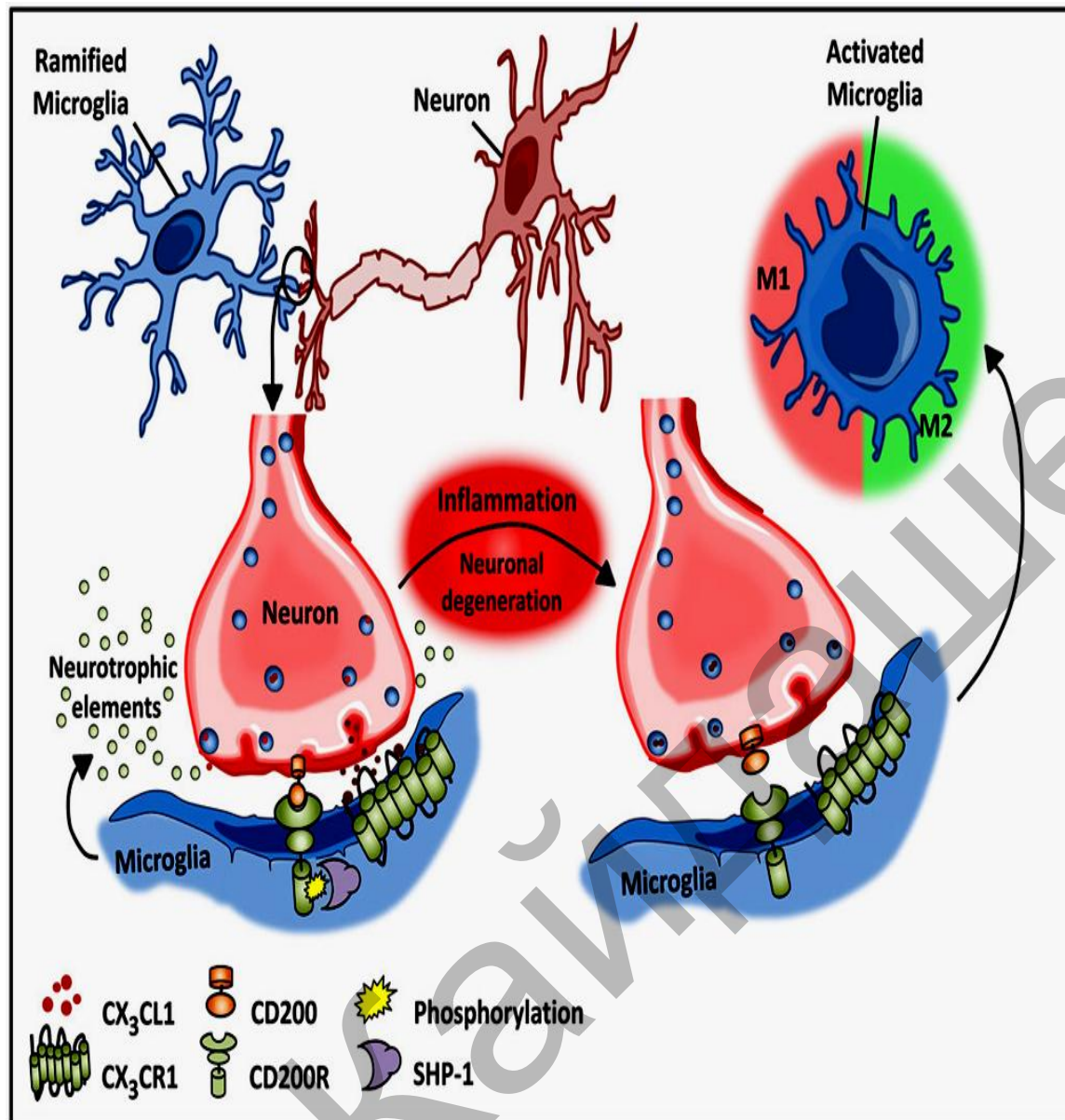


# Resident macrophage populations in steady state and inflammation



Resident microglia and Kupffer cell populations, both yolk sac-derived, are maintained through self-renewal, unlike macrophages resident to the adipose tissue, which are thought to originate from circulating blood monocytes (MO). Although the progression of tissue specific pathophysiological insults are dependent on both resident and infiltrating macrophages, monocyte-derived macrophages do not replenish resident microglia and Kupffer cell populations.

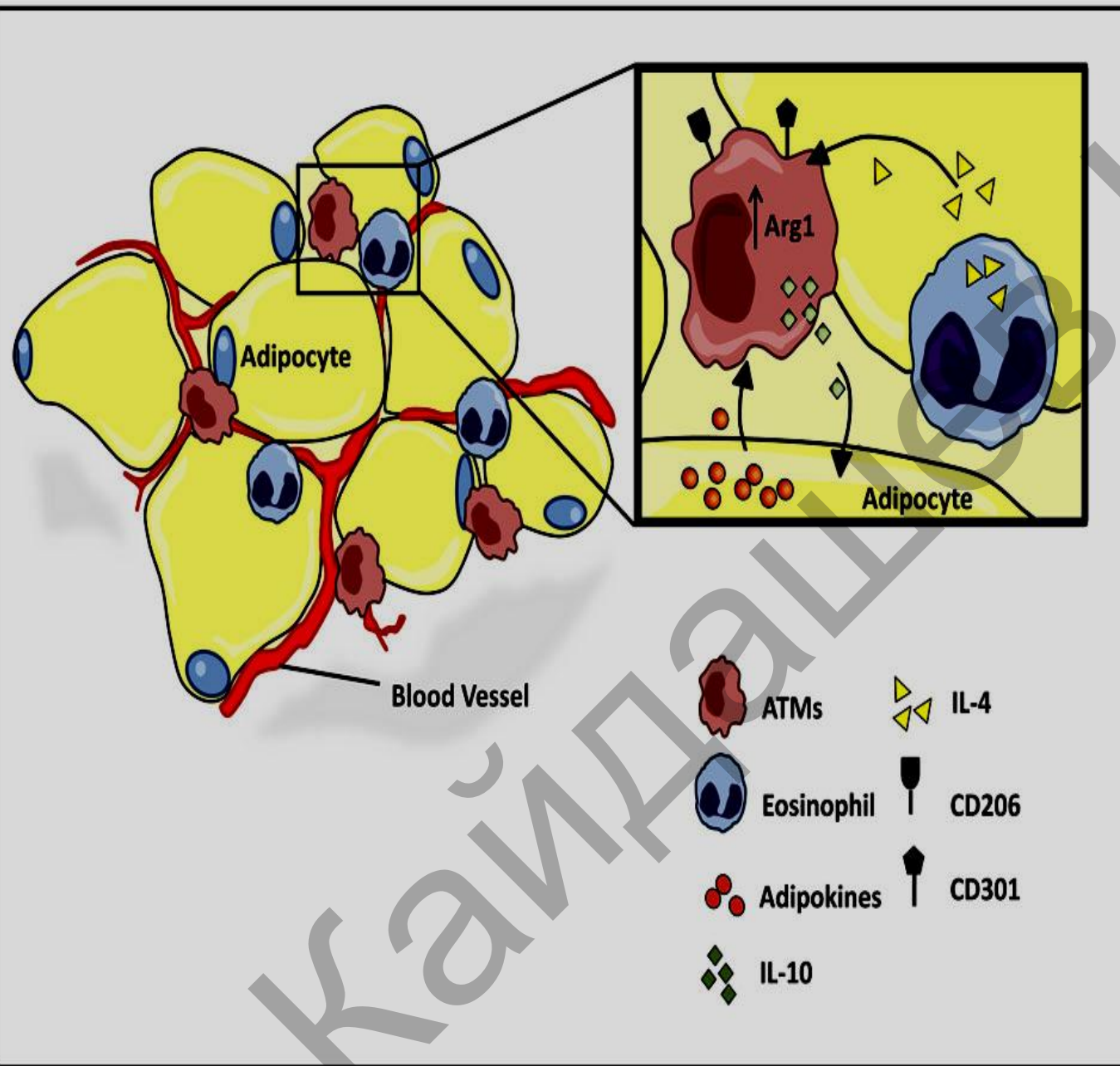
# Microglial–neuronal interactions in health and disease



Healthy neurons expressing chemokine fractalkine (CX3CL1) and CD200 membrane proteins intimately interact with their respective transmembrane protein receptors on microglia, CX3CR1, and CD200R to sustain a down-regulated microglial phenotype. Microglial receptors have immunoreceptor tyrosine-based inhibitory motifs (ITIMs), which upon ligand–receptor activation suppresses downstream immune signaling through the recruitment of phosphatases including SHP-1. Chronic inflammation disrupts this intimate neuronal–glial interaction, thus releasing the microglial cells from a down-regulated inhibited state to an activated phenotype.



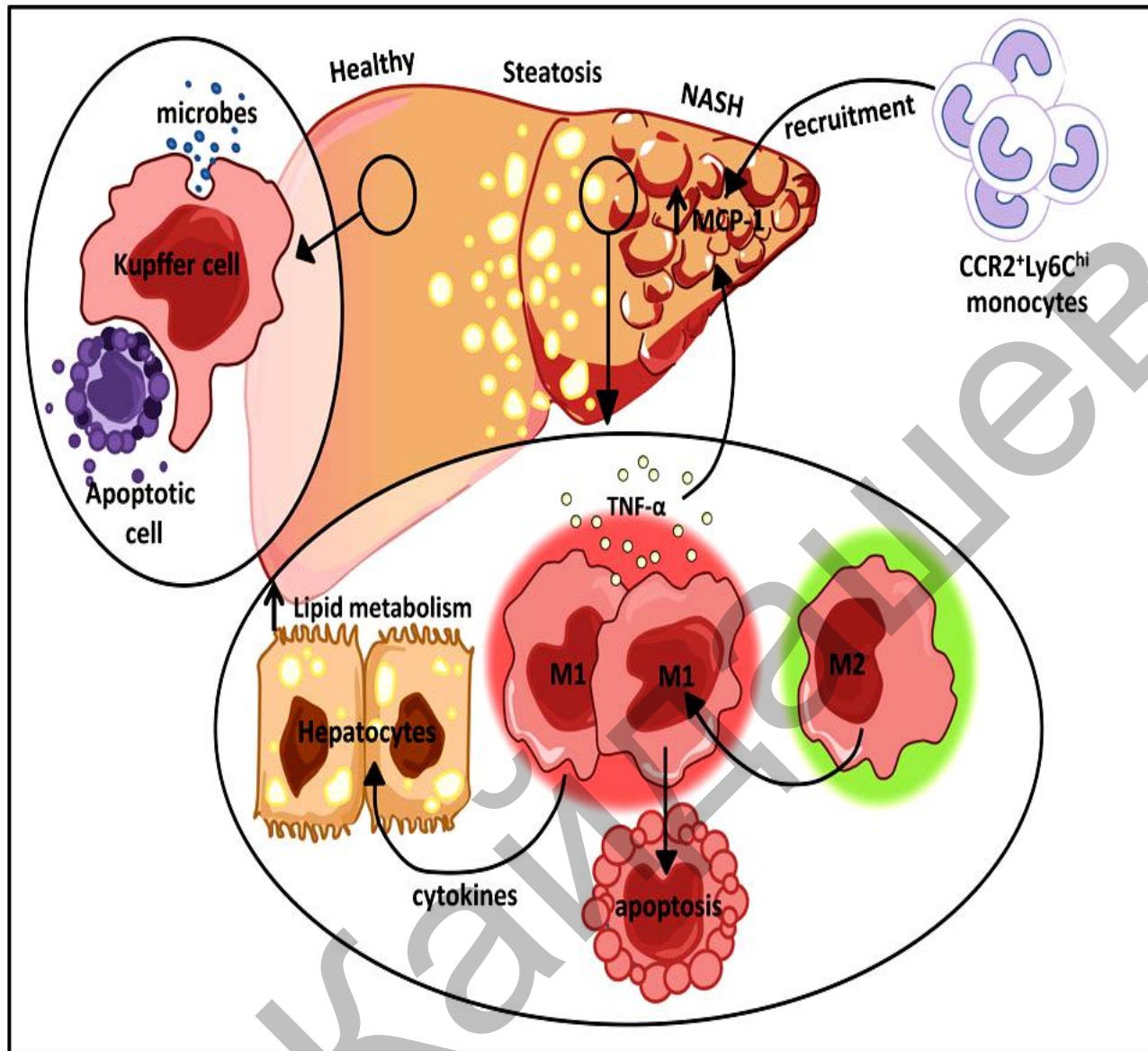
# Homeostatic regulation of ATM microenvironment



Healthy adipose tissue contains a relatively low and uniformly dispersed population of alternatively activated M2 macrophages, expressing cell surface antigens CD206 and CD301. The M2 polarized state is maintained by eosinophil and adipocyte derived adipokine secretions, IL-4, and adiponectin, respectively. M2 ATMs maintain a homeostatic adipose milieu with IL-10 secretions, which in turn regulate glucose homeostasis within systemic tissues.

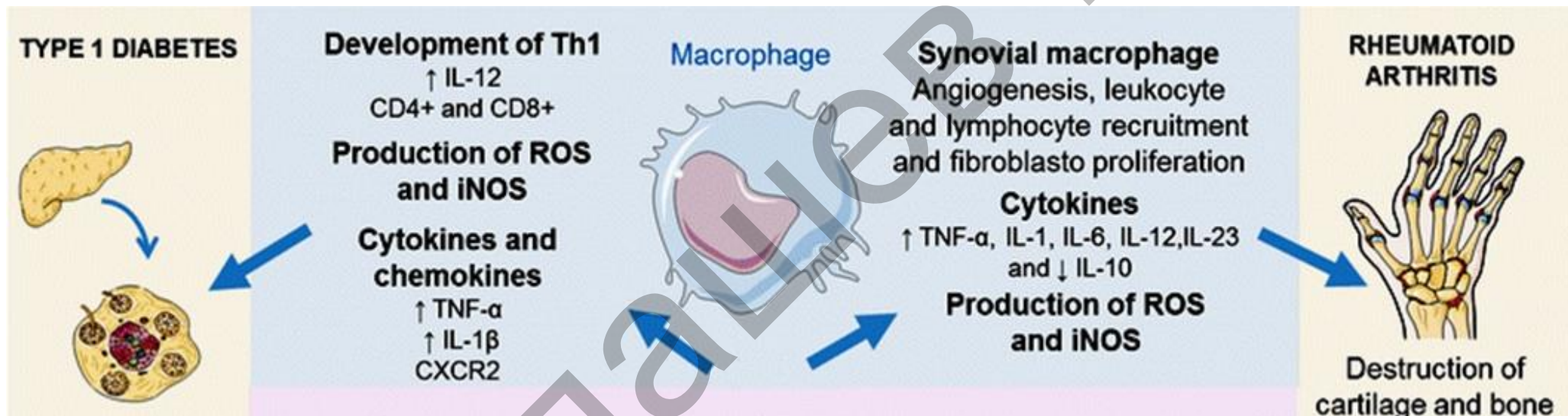


# The dual role of M1/M2 Kupffer cells in NAFLD

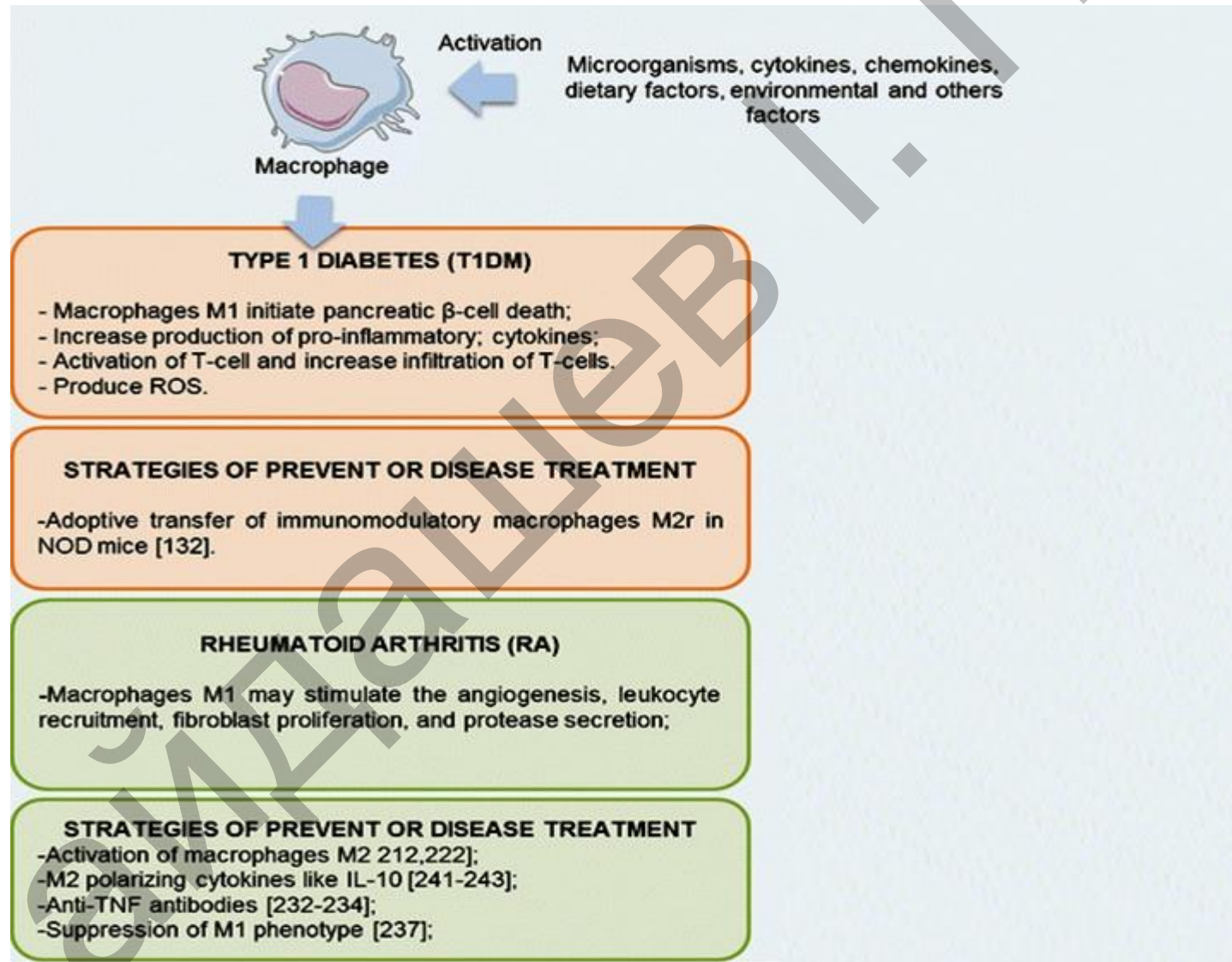


Kupffer cells play a pivotal role in host defense where they routinely clear microbes and apoptotic bodies from portal circulation. During diet-induced liver injury, tissue-resident macrophages exhibiting a classically activated M1 phenotype predominate and secrete cytokines that can alter hepatocyte lipid metabolism and induce MCP-1 dependent recruitment of monocytes into the liver. In turn, these infiltrating cells facilitate the development and progression of NAFLD. Restrained induction of M1 Kupffer cell apoptosis further perpetuates liver inflammation.

# Major mechanism of action of neutrophils and macrophages in type 1 diabetes and rheumatoid arthritis

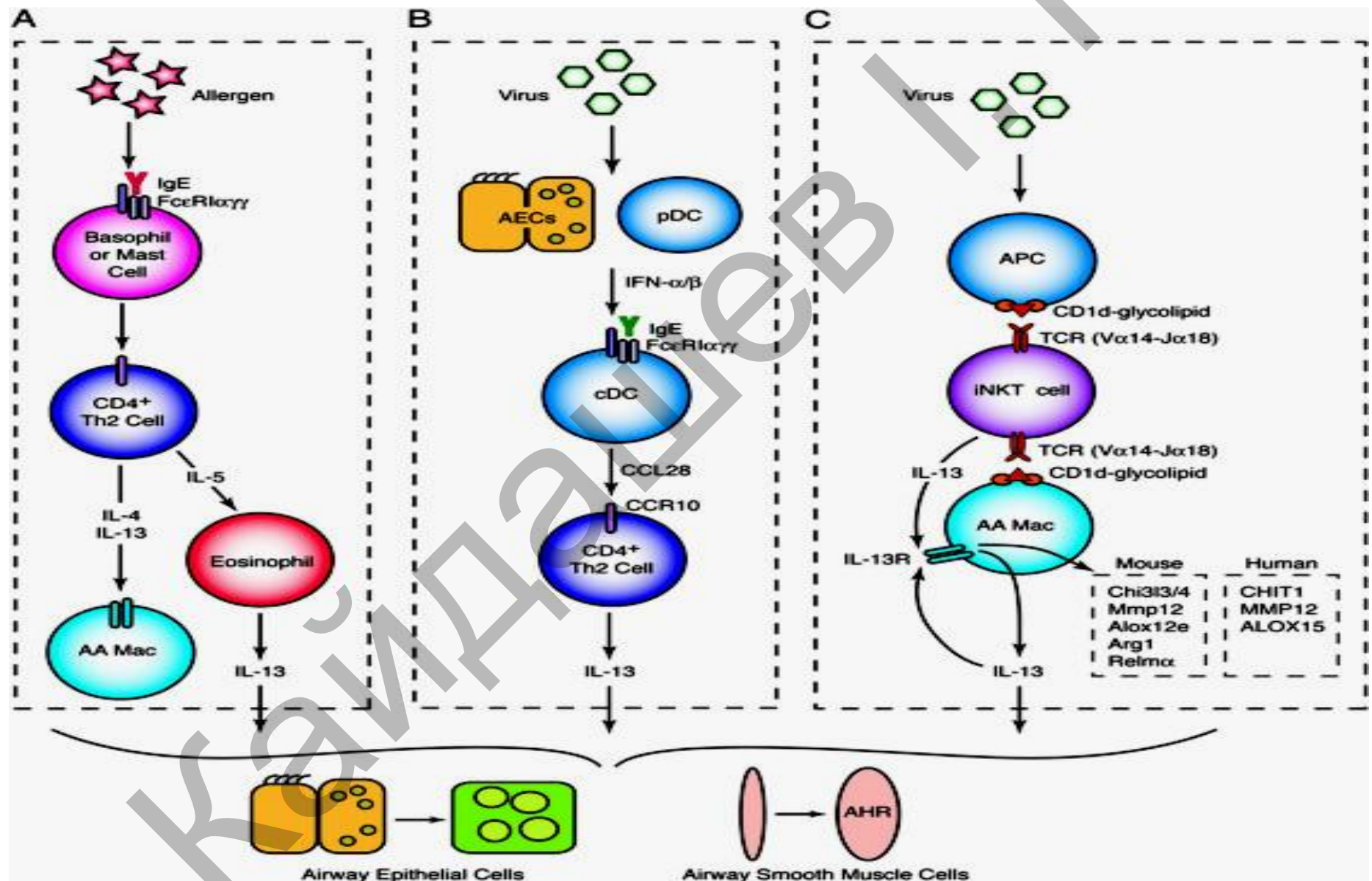


# A simplified schematic of the role of macrophage/neutrophil in autoimmune diseases type 1 diabetes and rheumatoid arthritis

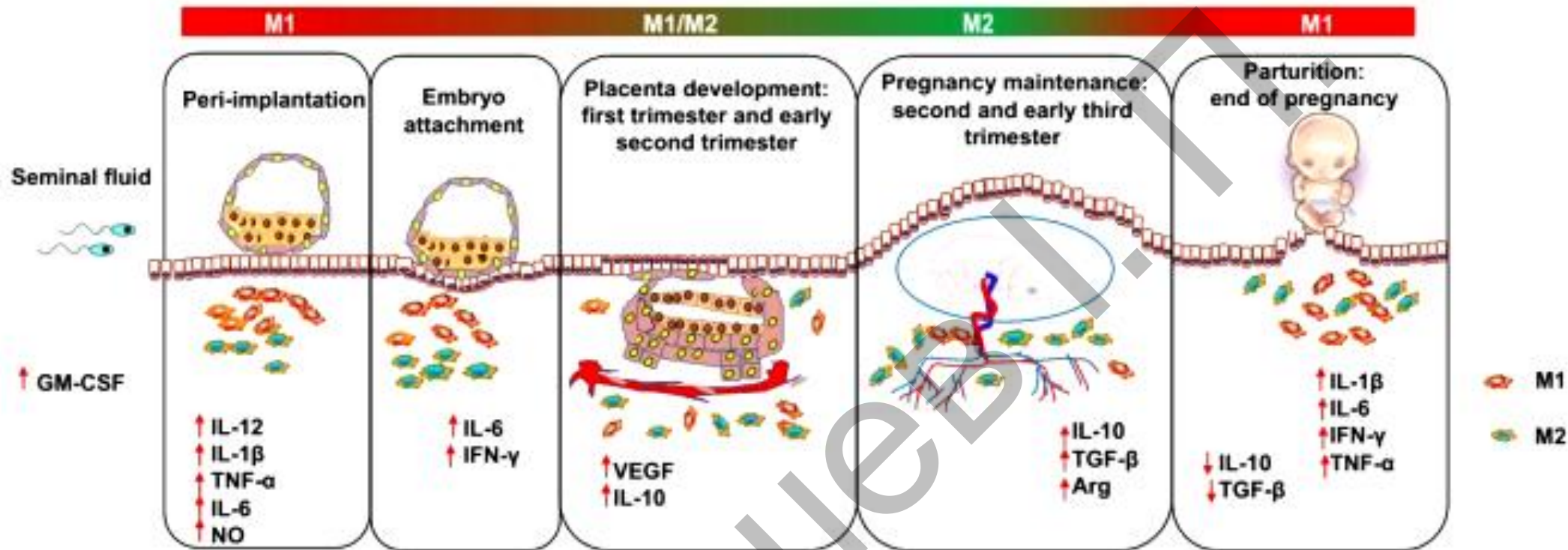




# Scheme for immune pathways leading to acute and chronic lung disease after viral infection or allergen challenge

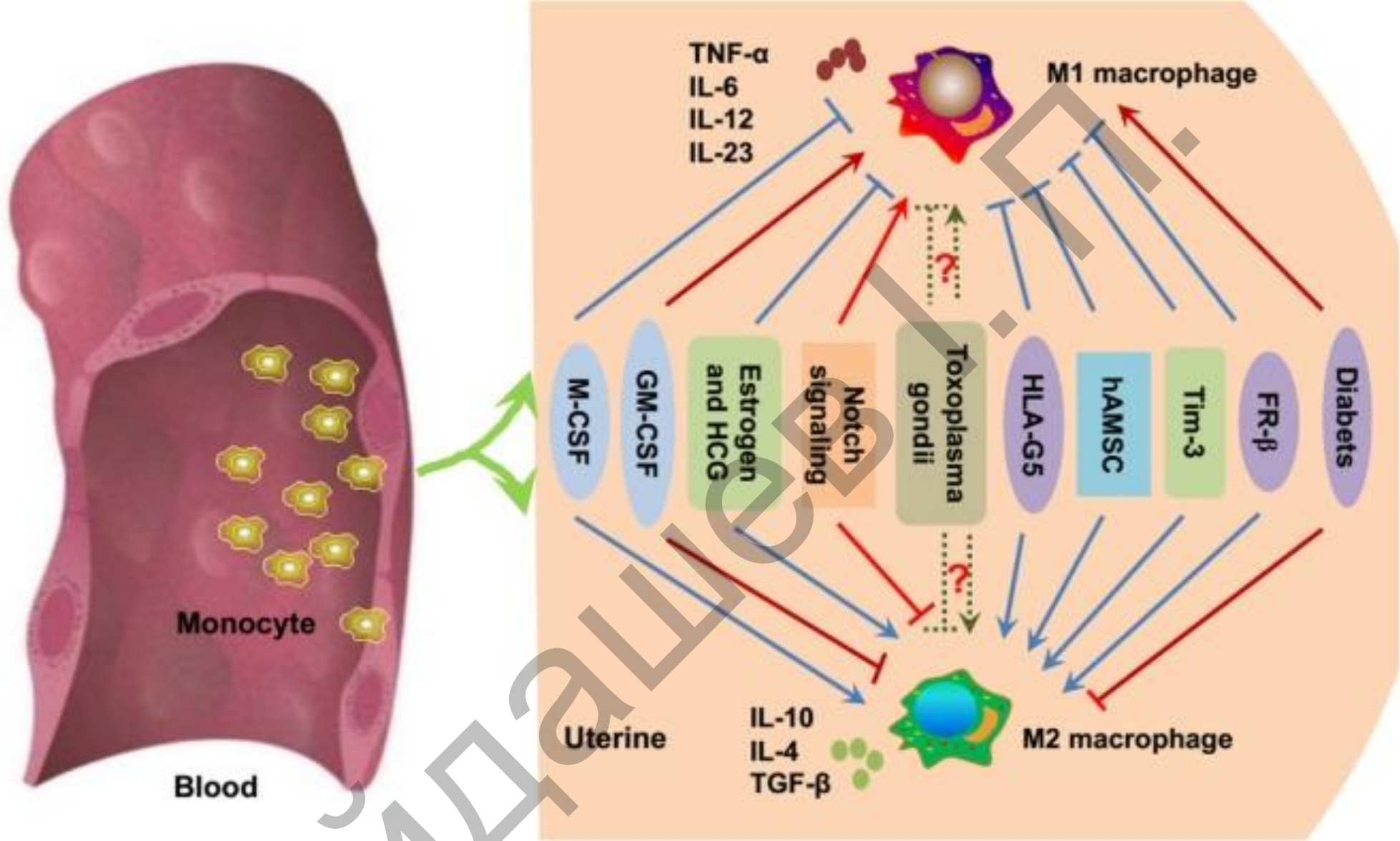


## Macrophage polarization during pregnancy



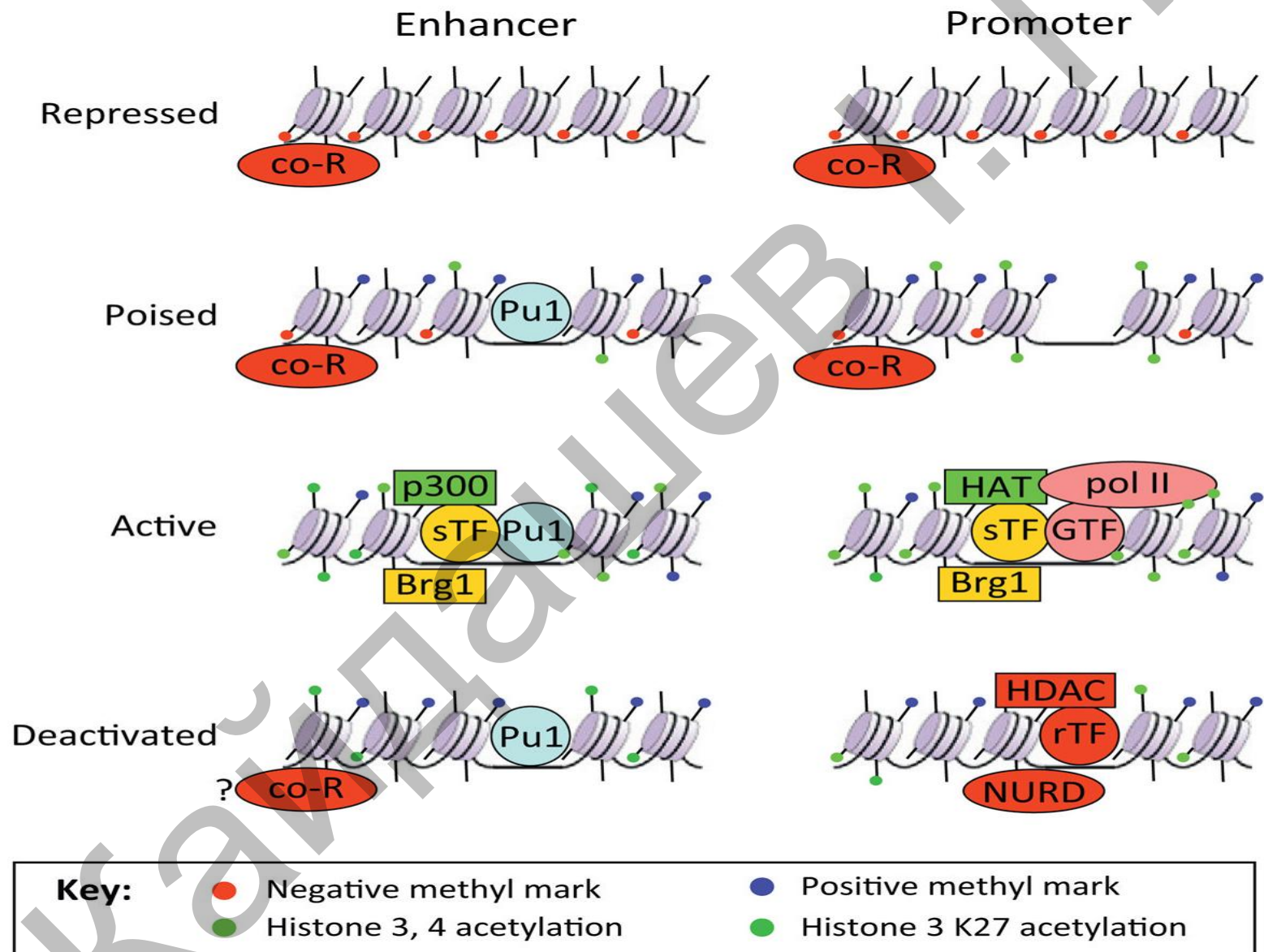
**Dynamics between M1 and M2 macrophages along pregnancy.** During the different phases of gestation, macrophages undergo dynamic changes, predominantly displaying the M1 or M2 phenotype. After coitus, granulocyte macrophage colony-stimulating factor levels are increased by transforming growth factor beta (TGF-β) in the seminal fluid and promote M1 activation. In the peri-implantation period, activated M1 macrophages produce inflammatory cytokines and mediators, such as interleukin (IL)-6, IL-1β, tumor necrosis factor alpha, and nitric oxide, inducing pro-inflammatory responses and promoting embryo attachment to the decidua. As the trophoblast invades the uterine stroma, decidual macrophages initiate an M1/M2 profile until the early phase of the second trimester of pregnancy, displaying both the pro- and anti-inflammatory phenotype, which endows the host with the ability to promote trophoblast invasion and vascular remodeling and prevent rejection of the embryo. Subsequently, in order to allow fetal development, more progesterone is produced, and an M2-dominant environment is established in the uterus until the end of pregnancy, which includes downregulation of inflammatory mediators, increased generation of anti-inflammatory cytokines (e.g., IL-10 and TGF-β), and phagocytosis of apoptotic debris. Finally, M1 macrophages predominate over the M2 subset again during the period of parturition, which is considered an inflammatory event. Accumulated M1 macrophages promote the contraction of the uterus, expulsion of the baby, ejection of the placenta and uterine involution.

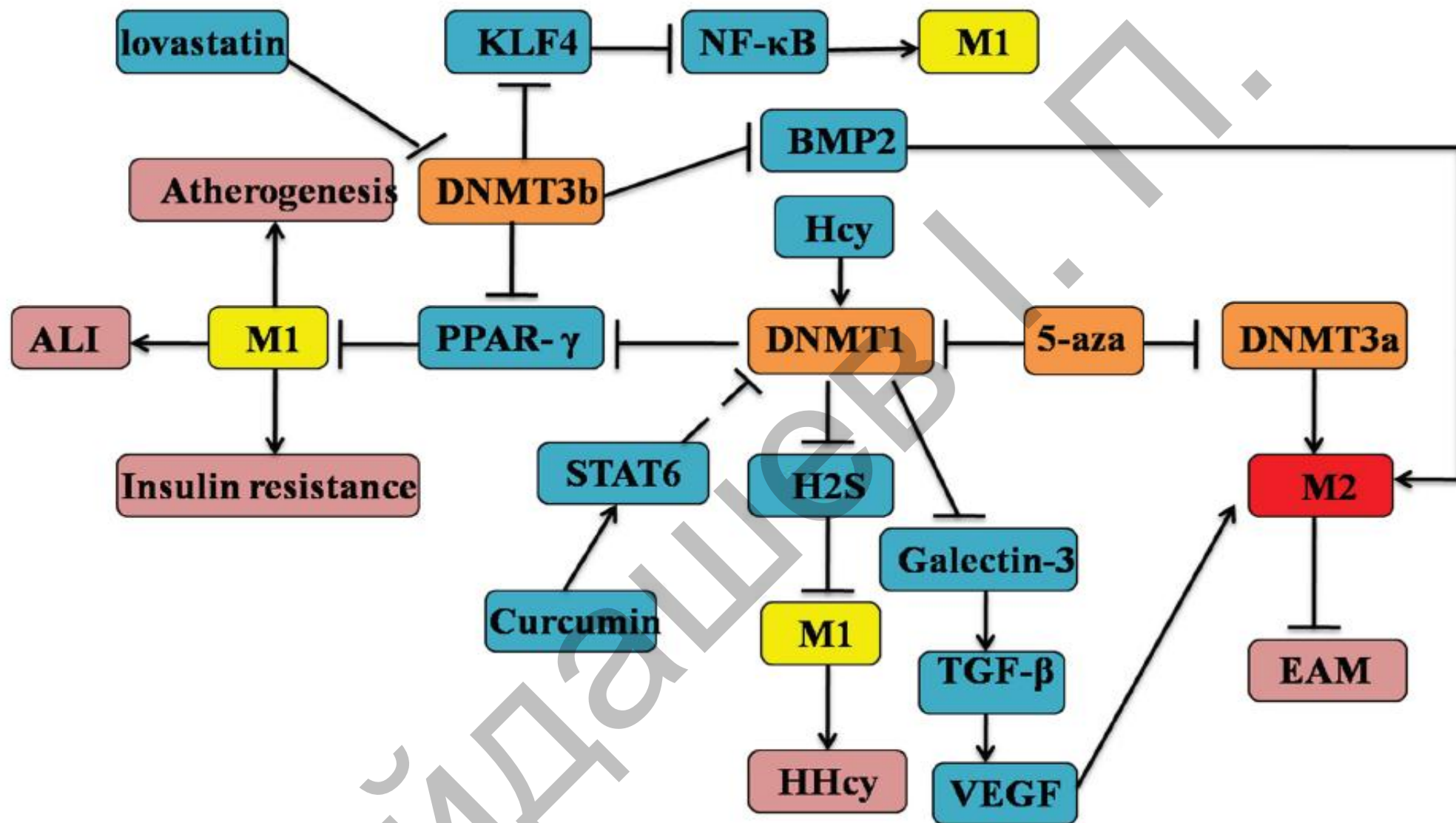




**Essential modulators of macrophage polarization during pregnancy.** Under inflammatory conditions, bone marrow-derived monocytes contribute to tissue macrophage homeostasis. As previously mentioned, M-CSF, estrogen, HCG, HLA-G5, hAMSC, Tim-3, and FR- $\beta$  promote the polarization toward M2 macrophages (blue point arrows) and inhibit M1 polarization (blue block arrows). GM-CSF, Notch signaling, and diabetes/hyperglycemia have been implicated in the polarization of M1 macrophages (red point arrows), while suppressing M2 macrophage polarization (red block arrows). Whether *Toxoplasma gondii* facilitates M1 or M2 macrophage polarization is uncertain (dashed green arrows), and it mainly depends on the host immune status and the virulence of the pathogen.

# Epigenetic regulation of inflammatory cytokine gene loci in macrophages

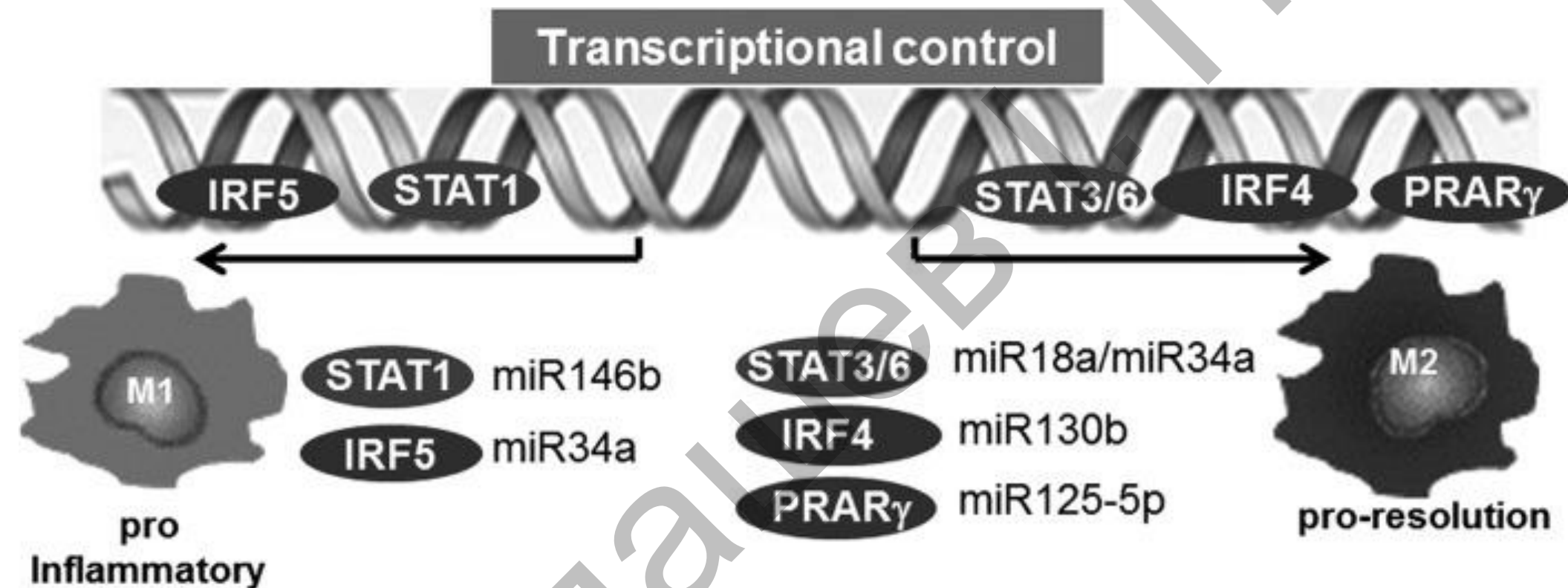




**Figure 3: DNA methylation in the determination of macrophages M1/M2 polarization.** Firstly, DNMTs are responsible for catalyzing epigenetic silencing and inappropriate activation of gene expression involved with the macrophage phenotypic changes. Then, DNMTs (including DNMT1, 3a and 3b) are differentially expressed in M1 or M2 macrophages, which might play opposite roles in the M1/M2 polarization. For example, the activation of DNMT1/3 might lead to the M1 polarization by targeting KLF4 and NF-κB signaling, which could be inhibited by 5-aza. Conversely, it inhibits the M2 macrophage polarization via the disturbance of the TGF-β and VEGF signaling. Among this complex process, STAT6 and other genes also participate in it.

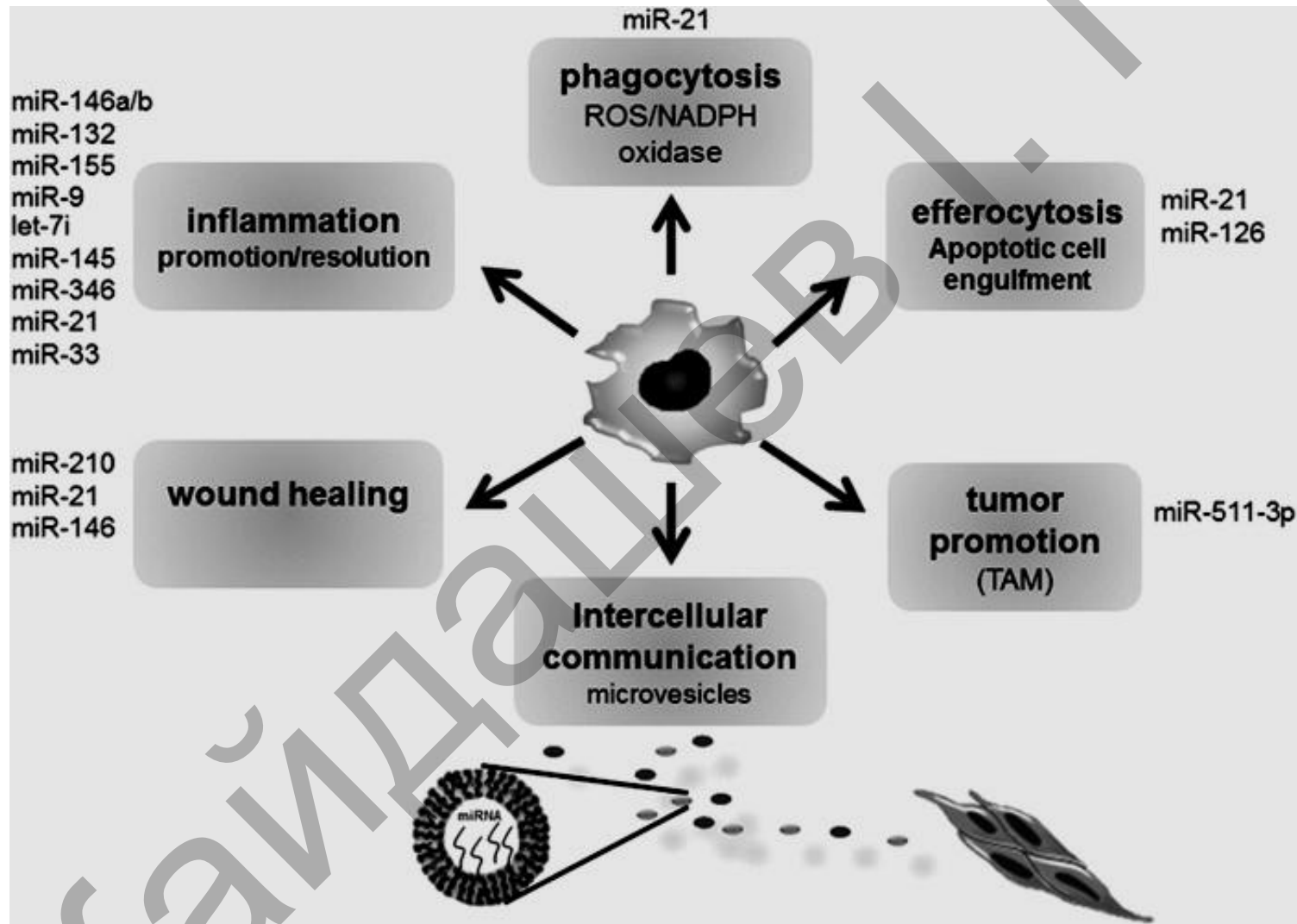


# Transcriptional control of macrophage polarization: role of miRNA



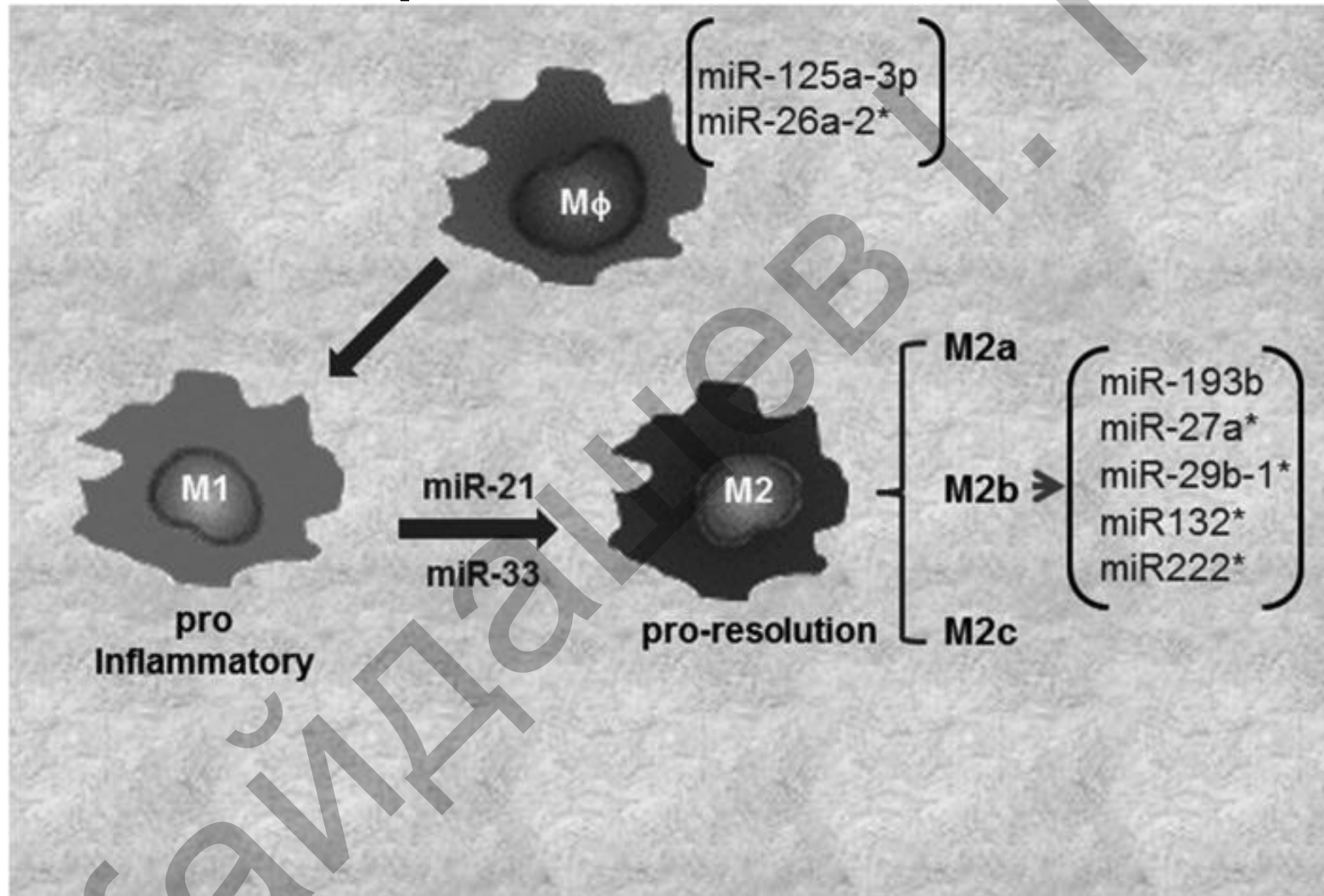
The microenvironmental signals elicit a transcriptional response that regulates the phenotype and function of the macrophages. The signal transducer and activator of transcription 1 (STAT1) and interferon regulatory factor 5 (IRF5) play a major role in dictating M1 macrophage phenotype, whereas, STAT3/STAT6, peroxisome proliferator-activated receptor- $\gamma$  (PPAR $\gamma$ ) and IRF4 direct M2 macrophage polarization. Major reported miRNAs that control these major transcription factors involved in macrophage polarization have been presented.

# miRNAs in macrophage function and intercellular communication



Macrophages are closely involved with a number of biological functions. Major miRNA that has been identified with each of the macrophage-mediated key process has been presented.

# Involvement of miRNAs in macrophage polarization



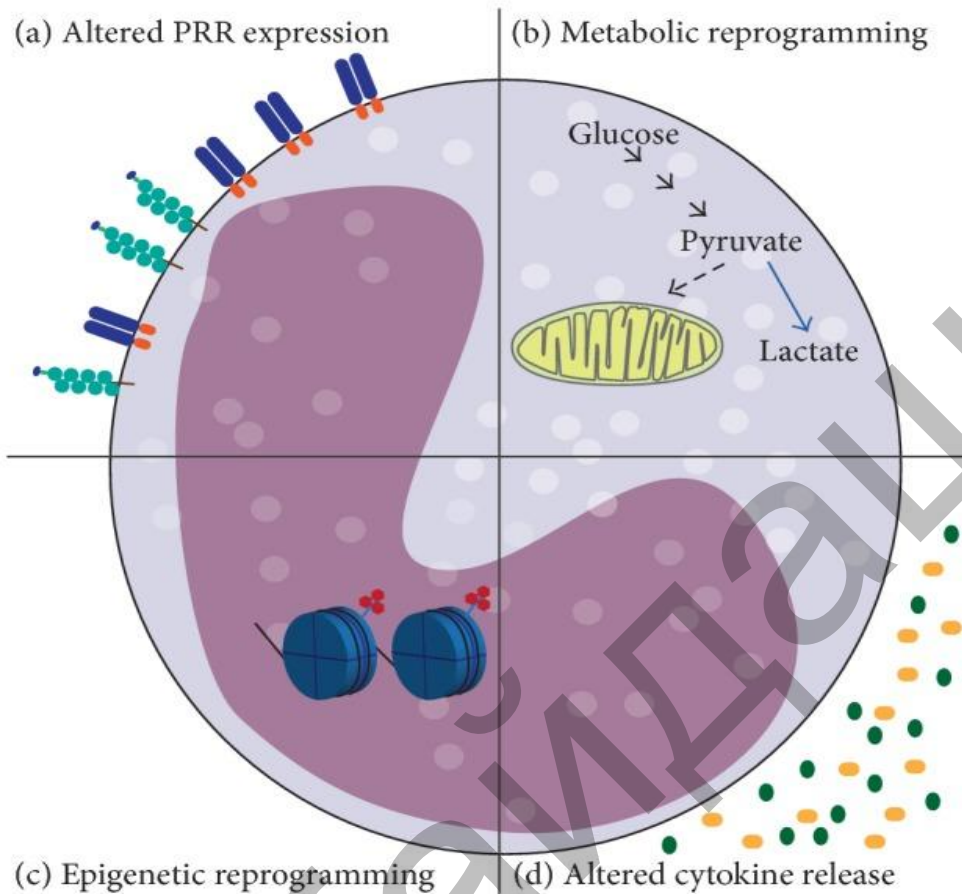
In tissues based on microenvironment, the macrophages get polarized to M1 or M2 (M2a, M2b, or M2c subtypes). Major miRNAs involved/expressed in macrophage polarization have been shown.



# Memory status of M $\phi$

- The molecular mechanism responsible for shifting M $\phi$  towards a memory status have not yet been elucidated.
- Probably underlain by epigenetic reprogramming (DNA modification, histone methylation, microRNA, and regulation of gene expression by inducing dynamic alterations in chromatin structure).
- Efficacy of many vaccines probably implies the induction of non-specific macrophage memory that contributes to the increased resistance to infections.

# Main mechanisms involved in trained immune memory



- (a) Altered PRR expression. Phenotypic changes of innate immune cells with memory properties involve increased expression of PRRs on the cell surface and improved pathogen recognition.
- b) Metabolic reprogramming. Innate immune memory requires a metabolic shift, which involves Warburg metabolism. The metabolism of glucose is shifted toward increased glycolysis with production of lactate and decreased oxidative phosphorylation.
- (c) Epigenetic reprogramming. Trimethylation of H3 at lysine 4 (H3K4me3) is a marker of promoter activation for proinflammatory genes specifically induced by  $\beta$ -glucan-dependent memory.
- (d) Altered cytokines release.

# **Revolutionary concepts on M $\phi$ that should force the researcher to rewrite the books of immunology**

- The embryonic origin of tissue-resident M $\phi$ .
- The capacity of Mo/M $\phi$  to polarize into distinct functional phenotype.
- The notion of innate memory (so-called trained innate immunity).
- The importance of macrophage-mediated antigen presentation in tissue responses.
- M $\phi$  have a major role in inducing and modulating adaptive immunity (including the induction of polarized T cell responses).

